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Compositions from the grasses Lolium perenne and Festuca arundinacea.

COMPOSITIONS ISOLATED FROM FORAGE GRASSES AND METHODS FOR THEIR USE

Reference to Related Applications

This application claims priority to U.S. Provisional Patent Application No. 60/337,703 filed November 7, 2001.

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Technical Field of the Invention

This invention relates to polynucleotides isolated from forage grass tissues, specifically from *Lolium perenne* (perennial ryegrass) and *Festuca arundinacea* (tall fescue), as well as oligonucleotide probes and primers, genetic constructs comprising the polynucleotides, biological materials (including host cells and plants) incorporating the polynucleotides, polypeptides encoded by the polynucleotides, and methods for using the polynucleotides and polypeptides. More particularly, the invention relates to polypeptides involved in the lignin, tannin and fructan biosynthetic pathways, and to polynucleotides encoding such polypeptides.

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Background of the Invention

Over the past 50 years, there have been substantial improvements in the genetic production potential of ruminant animals (sheep, cattle and deer). Levels of meat, milk or fiber production that equal an animal's genetic potential may be attained within controlled feeding systems, where animals are fully fed with energy dense, conserved forages and grains. However, the majority of temperate farming systems worldwide rely on the *in situ* grazing of pastures. Nutritional constraints associated with temperate pastures can prevent the full expression of an animal's genetic potential. This is illustrated by a comparison between milk production by North American grain-fed dairy cows and New Zealand pasture-fed cattle. North American dairy cattle produce, on average, twice the milk volume of New Zealand cattle, yet the genetic base is similar within both systems (New Zealand Dairy Board and United States Department of Agriculture figures). Significant potential therefore exists

to improve the efficiency of conversion of pasture nutrients to animal products through the correction of nutritional constraints associated with pastures.

Lignin Biosynthetic Pathway

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Lignin is an insoluble polymer that serves as a matrix around the polysaccharide components of some plant cell walls, and that is primarily responsible for the rigidity of plant stems. Generally, the higher the lignin content, the more rigid the plant. For example, tree species synthesize large quantities of lignin, with lignin constituting 20%-30% of the dry weight of wood. The lignin content of grasses ranges from 2-8% of dry weight and changes during the growing season. In addition to providing rigidity, lignin aids in water transport within plants by rendering cell walls hydrophobic and water impermeable. Lignin also plays a role in disease resistance of plants by impeding the penetration and propagation of pathogenic agents.

Forage digestibility is affected by both lignin composition and concentration. Lignin is largely responsible for the digestibility, or lack thereof, of forage crops, with small increases in plant lignin content resulting in relatively high decreases (> 10%) in digestibility (Buxton and Russell, *Crop. Sci.* 28:5358-558, 1988). For example, crops with reduced lignin content provide more efficient forage for cattle, with the yield of milk and meat being higher relative to the amount of forage crop consumed. During normal plant growth, an increase in the maturity of the plant stem is accompanied by a corresponding increase in lignin content and composition that causes a decrease in digestibility. This change in lignin composition is to one of increasing syringyl:guaiacyl (S:G) ratio. When deciding on the optimum time to harvest forage crops, farmers must therefore choose between a high yield of less digestible material and a lower yield of more digestible material.

Lignin is formed by polymerization of three different monolignols, *para*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol, that are synthesized in a multistep pathway, with each step in the pathway being catalyzed by a different enzyme. The three monolignols are derived from phenylalanine or tyrosine in a multistep process and are then polymerized into lignin by a free radical mechanism. Following polymerization, *para*-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol are converted into the *p*-hydroxyphenyl (H), guaiacyl (G) and syringyl (S) units of lignin, respectively. While these three types of lignin subunits

are well known, it is likely that slightly different variants of these subunits may be involved in the lignin biosynthetic pathway in various plants. For example, studies suggest that both free monolignols and monolignol-4-coumarate esters may be substrates for lignin formation in grasses. The relative concentration of the monolignol residues in lignin varies among different plant species and within species. For example, the monolignol content for H/G/S of grasses, alfalfa and softwood gymnosperms is 22%/44%/34%, 7%/39%/54% and 14%/80%/6%, respectively (van Soest in "Nutritional Ecology of the Ruminant". Cornell University Press, Ithaca, NY). The composition of lignin may also vary among different tissues within a specific plant.

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Coniferyl alcohol, para-coumaryl alcohol and sinapyl alcohol are synthesized by similar pathways (Whetten et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:585-609, 1998; Guo et al., Plant Cell 13:73-88, 2001). The first step in the lignin biosynthetic pathway is the deamination of phenylalanine or tyrosine by phenylalanine ammonia-lyase (PAL) or tyrosine ammonia-lyase (TAL), respectively. In maize, the PAL enzyme also has TAL activity (Rosler et al., Plant Physiol. 113:175-179, 1997). The product of TAL activity on tyrosine is 4-coumarate, whereas the product of PAL activity on phenylalanine is cinnamate which is then hydroxylated by cinnamate 4-hydroxylase (C4H) to form 4-4-Coumarate is hydroxylated by coumarate 3-hydroxylase (C3H) to give coumarate. caffeate. The newly added hydroxyl group is then methylated by caffeic acid O-methyl transferase (COMT) to give ferulate. Several other methylation reactions can be catalyzed by COMT, including caffeoylaldehyde to coniferaldehyde, and 5-hydroxyconiferaldehyde to sinapaldehyde. 4-Coumarate, caffeate and ferulate can all be conjugated to coenzyme A by 4-coumarate: CoA ligase (4CL) to form 4-coumaryl CoA, caffeoyl CoA and feruloyl CoA, respectively. Caffeoyl CoA can then be methylated by the enzyme caffeoyl-CoA O-methyl transferase (CAMT).

Coniferaldehyde is hydroxylated to 5-hydroxyconiferaldehyde by ferulate 5-hydroxylase (F5H). Reduction of 4-coumaryl CoA, caffeoyl CoA and feruloyl-CoA to 4-coumaraldehyde, caffeoyl aldehyde and coniferaldehyde, respectively, is catalyzed by cinnamoyl-CoA reductase (CCR). Coumaraldehyde, caffeoyl aldehyde, coniferaldehyde and 5-hydroxyconfieraldehyde are further reduced by the action of cinnamyl alcohol dehydrogenase (CAD) to give coniferyl alcohol which is then converted into its glucosylated

form for export from the cytoplasm to the cell wall by coniferol glucosyl transferase (CGT). Recently a sinapyl alcohol dehydrogenase (SAD) was described that converts sinapaldehyde to sinapyl alcohol (Li et al., Plant Cell 13:1567-1586, 2001). Following export, the deglucosylated form of coniferyl alcohol is obtained by the action of coniferin beta-glucosidase (CBG). Finally, polymerization of the three monolignols to provide lignin is catalyzed by phenolase (PNL), laccase (LAC) and peroxidase (PER). For a more detailed review of the lignin biosynthetic pathway, see Whetton R and Sederoff R, The Plant Cell, 7:1001-1013, 1995 and Whetten et al., Annu. Rev. Plant Physiol. Plant Mol. Biol. 49:585-609, 1998.

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Both lignin levels and composition have been changed in a range of plant species by altering the expression of specific lignin biosynthetic enzymes. For example, anti-sense 4CL constructs in transgenic aspen trees reduced lignin content from 20 to 11% (a 45% reduction) but at the same time increased both cellulose levels (by 15%) and growth rate (Hu et al. Nature Biotechnol. 17:808-812, 1999). These trees had the same level of total carbon, suggesting that carbon partitioning had been altered. Reducing 4CL by either anti-sense or sense-suppression in tobacco plants led to an accumulation of hydroxycinnamic acids in cell walls as well as a reduction in both guaiacyl and syringyl lignin units (Kajita et al., Plant Cell. Physiol. 37:957-965, 1996). In transgenic tobacco plants in which levels of C4H were reduced by anti-sense or sense suppression, total lignin content was reduced, in addition to a reduction in syringyl lignin units (Sewalt et al., Plant Physiol. 115:41-50, 1997). Reducing the levels of PAL in tobacco plants by anti-sense or sense-suppression reduced total lignin content but did not change the syringyl-guaiacyl (S:G) lignin ration. In alfalfa, reducing expression of COMT through either anti-sense or gene silencing decreased total lignin by decreasing the amount of guaiacyl units and resulted in a near total loss of syringyl lignin units (Guo et al., Plant Cell 13:73-88, 2001). In contrast, reducing CCOMT expression through anti-sense or gene silencing in alfalfa plants also decreased total lignin by reducing the total amount of guaiacyl lignin units but had no effect on the amount of syringyl lignin. Reducing CCR expression by anti-sense in tobacco plants resulted in reduced lignin content and increased S:G ratios due to lower guaiacyl lignin units (Piquemal et al., Plant J. 13:71-83, 1998). A. thaliana plants where the F5H gene had been mutated contained only traces of syringyl lignin (Marita et al., Proc. Natl. Acad. Sci. USA 96:12323-12332, 1999).

Alteration of grass lignin composition may usefully be employed to maintain high forage digestibility throughout the year. This is most important when the plant is approaching flowering and/or during flowering. At this time, the entire lignin biosynthetic pathway will preferably be reduced, in particular lowering the amount of syringyl lignin units, thereby lowering the S:G ratio and maintaining the digestibility of the forage crop.

Several of the enzymes involved in the lignin biosynthetic pathway also have other functions within the plant. For example, PAL is a key enzyme of plant and fungi phenylpropanoid metabolism and catalyzes the first step in phenylpropanoid metabolism. It is involved in the biosynthesis of a wide variety of secondary metabolites such as flavonoids, furanocoumar in phytoalexins and cell wall components. These compounds have many important roles in plants during normal growth and in responses to environmental stress. PAL catalyzes the removal of an ammonia group from phenylalanine to form transcinnamate. PAL and the related histidine ammonia lyase are unique enzymes which are known to have the modified amino acid dehydroalanine (DHA) in their active site (Taylor et al., J. Biol. Chem. 265:18192-18199, 1990). Phenylalanine and histidine ammonia-lyases (PAL) active site has a consensus of GTITASGDLVPLSYIA. The serine residue is central to the active site, and the region around this active site residue is well conserved (Langer et al., Biochem. 33:6462-6467, 1994).

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C4H, which is a member of the cytochrome P450 monooxygenase superfamily, plays a central role in both phenylpropanoid metabolism and lignin biosynthesis where it anchors a phenylpropanoid enzyme complex to the endoplasmic reticulum (ER). The phenylpropanoid pathway controls the synthesis of lignin, flower pigments, signaling molecules, and a large spectrum of compounds involved in plant defense against pathogens and UV light. This is also a branch point between general phenylpropanoid metabolism and pathways leading to various specific end products. 4CLs are a group of enzymes necessary for maintaining a continuous metabolic flux for the biosynthesis of plant phenylpropanoids, such as lignin and flavonoids that are essential to the survival of plants, because they serve important functions in plant growth and adaptation to environmental perturbations. Three isoforms of 4CL have been identified with distinct substrate preference and specificities. Expression studies in angiosperms revealed a differential behavior of the three genes in various plant organs and upon external stimuli such as wounding and UV irradiation or upon challenge with fungi.

One isoform is likely to participate in the biosynthetic pathway leading to flavonoids whereas the other two are probably involved in lignin formation and in the production of additional phenolic compounds other than flavonoids (Ehlting *et al.*, *Plant J.* 19:9-20, 1999).

F5H is involved in the phenylpropanoid biosynthesis pathway. It belongs to the CYP84 subfamily of the cytochrome P450 family and is known as cytochrome P450 84A1. F5H is one of the enzymes in the pathways leading to the synthesis of sinapic acid esters, but also has coniferaldehyde hydroxylase activity (Nair et al., Plant Physiol. 123:1623-1634, 2000). In the generalized pathway for phenylpropanoid metabolism, F5H catalyzes the formation of 5-hydoxyferulate (a precursor of sinapate) and sinapate in turn as the precursor for sinapine and for sinapoyl CoA in two bifurcated pathways (Chapple et al., Plant Cell 4:1413-1424, 1992). Sinapoyl CoA has been considered as the precursor for sinapyl alcohol, which is then polymerized into syringyl (S) lignin. In addition, CYP84 F5H product carries out the hydroxylation of coniferaldehyde (ConAld) to 5-OH ConAld (Nair et al., Plant Physiol. 123:1623-1634, 2000).

Peroxidases are heme-containing enzymes that use hydrogen peroxide as the electron acceptor to catalyze a number of oxidative reactions. They belong to a superfamily consisting of 3 major classes. Class III consists of the secretory plant peroxidases, which have multiple tissue-specific functions in removal of hydrogen peroxide from chloroplasts and cytosol, oxidation of toxic compounds, biosynthesis of the cell wall, defense responses towards wounding, indole-3-acetic acid (IAA) catabolism and ethylene biosynthesis.

Fructan Biosynthetic Pathway

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Plant carbohydrates can be divided into two groups depending on their function within the plant. Structural carbohydrates, such as cellulose, are usually part of the extracellular matrix. Non-structural, storage carbohydrates act as either long- or short-term carbohydrate stores. Examples of non-structural carbohydrates include starch, sucrose and fructans.

Fructans are polymers that are stored in the vacuole and that consist of linear and branched chains of fructose units (for review see Vijn and Smeekens *Plant Physiol.* 120:351-359, 1999). They play an important role in assimilate partitioning and possibly in stress tolerance in many plant families. Grasses use fructans instead of starch as a water-soluble

carbohydrate store (Pollock et al., in "Regulation of primary metabolic pathways in plants", N.J. Kruger et al., (eds), Kluwer Academic Publishers, The Netherlands, pp195-226, 1999). Increasing the amount of fructans and sucrose in forage crops leads to an increase in the level of water-soluble carbohydrates and thereby enhances the nutritional value of the plants. In addition, increasing the amount of fructans in forage plants decreases methane production in animals fed the plants, thereby leading to lower greenhouse gas emissions, and decreases urea production in animals as less protein is degraded in the rumen (Biggs and Hancock, Trends in Plant Sci. 6:8-9, 2001). Fructans have also been implicated in protecting plants against water deficits caused by drought or low temperatures. Introduction of enzymes involved in the fructan biosynthetic pathway into plants that do not naturally synthesize fructans may be employed to confer cold tolerance and drought tolerance (Pilon-Smits, Plant Physiol. 107:125-130, 1995).

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The number of fructose units within a fructan chain is referred to as the degree of polymerization (DP). In grasses, fructans of DP 6-10 are common. Such fructans of low DP are naturally sweet and are therefore of interest for use as sweeteners in foodstuffs. Long fructan chains form emulsions with a fat-like texture and a neutral taste. The human digestive system is unable to degrade fructans, and fructans of high DP may therefore be used as low-calorie food ingredients. Over-expression of enzymes involved in the fructan biosynthetic pathway may be usefully employed to produce quantities of fructans that can be purified for human consumption.

Five major classes of structurally different fructans have been identified in plants, with each class showing a different linkage of the fructosyl residues. Fructans found in grasses are of the mixed levan class, consisting of both (2-1)- and (2-6)-linked beta-D-fructosyl units (Pollock *et al.*, in "Regulation of primary metabolic pathways in plants", N.J. Kruger *et al.*, (eds), Kluwer Academic Publishers, The Netherlands, pp195-226, 1999). Fructans are synthesized by the action of fructosyltransferase enzymes on sucrose in the vacuole. These enzymes are closely related to invertases, enzymes that normally hydrolyze sucrose.

Grasses use two fructosyltransferase enzymes to synthesize fructans, namely sucrose:sucrose 1-fructosyltransferase (1-SST) and sucrose:fructan 6-fructosyltransferase (6-SFT) (Pollock *et al.*, in "Regulation of primary metabolic pathways in plants", N.J. Kruger *et*

al., (eds), Kluwer Academic Publishers, The Netherlands, pp195-226, 1999). 1-SST is a key enzyme in plant fructan biosynthesis, while 6-SFT catalyzes the formation and extension of beta-2,6-linked fructans that is typically found in grasses. Specifically, 1-SST catalyzes the formation of 1-kestose plus glucose from sucrose, while 6-SFT catalyzes the formation of bifurcose plus glucose from sucrose plus 1-kestose and also the formation of 6-kestose plus glucose from sucrose. Both enzymes can modify 1-kestose, 6-kestose and bifurcose further by adding additional fructose molecules. Over-expression of both 1-SST and 6-SFT in the same plant may be employed to produce fructans for use in human foodstuffs (Sevenier et al., Nature Biotechnol. 16:843-846; Hellwege et al., Proc. Nat. Acad. Sci. USA 97:8699-8704, 2000).

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The synthesis of sucrose from photosynthetic assimilates in plants, and therefore the availability of sucrose for use in fructan formation, is controlled, in part, by the enzymes sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP). Sucrose plays an important role in plant growth and development, and is a major end product of photosynthesis. It also functions as a primary transport sugar and in some cases as a direct or indirect regulator of gene expression (for a review see Smeekens, Curr. Opin. Plant Biol. 1:230-234, 1998). SPS regulates the synthesis of sucrose by regulating carbon partitioning in the leaves of plants and therefore plays a major role as a limiting factor in the export of photoassimilates out of the leaf. The activity of SPS is regulated by phosphorylation and moderated by concentration of metabolites and light (Huber et al., Plant Physiol. 95:291-297, 1991). Specifically, SPS catalyzes the transfer of glucose from UDP-glucose to fructose-6-Suc-6-P is then phosphate, thereby forming sucrose-6-phosphate (Suc-6-P). dephosphorylated by SPP to form sucrose (Lunn et al., Proc. Natl. Acad. Sci. USA 97:12914-12919, 2000). The enzymes SPS and SPP exist as a heterotetramer in the cytoplasm of mesophyll cells in leaves, with SPP functioning to regulate SPS activity. SPS is also important in ripening fruits, sprouting tubers and germinating seeds (Laporte et al., Planta 212:817-822, 2001).

Once in the vacuole, sucrose can be converted into fructan by fructosyltransferases as discussed above, or hydrolyzed into glucose and fructose by the hydrolase enzymes known as invertases (Sturm, *Plant Physiol.* 121:1-7, 1999). There are several different types of invertases, namely extracellular (cell wall), vacuolar (soluble acid) and cytoplasmic, with at

least two isoforms of each type of invertase normally being found within a plant species. In addition to having different subcellular locations, the different types of invertases have different biochemical properties. For example, soluble and cell wall invertases operate at acidic pH, whereas cytoplasmic invertases work at a more neutral or alkaline pH. Invertases are believed to regulate the entry of sucrose into different utilization pathways (Grof and Campbell, Aust. J. Plant Physiol. 28:1-12, 2001). Reduced invertase activity may increase the level of water-soluble carbohydrates in plants. Plants contain several isoforms of cell wall invertases (CWINV), which accumulate as soluble proteins. CWINV plays an important role in phloem unloading and in stress response. It hydrolyzes terminal non-reducing beta-D-fructofuranoside residues in beta-D-fructo-furanosides.

Another enzyme that acts upon sucrose in plants is soluble sucrose synthase (SUS). Recent results indicate that SUS is localized in the cytosol, associated with the plasma membrane and the actin cytoskeleton. Phosphorylation of SUS is one of the factors controlling localization of the enzyme (Winter and Huber, *Crit. Rev. Biochem. Mol. Biol.* 35:253-89, 2000). It catalyzes the transfer of glucose from sucrose to UDP, yielding UDP-glucose and fructose. Increasing the amount of SUS in a plant increases the amount of cellulose synthesis, whereas decreasing SUS activity should increase fructan levels. Increased SUS concentration may also increase the yield of fruiting bodies. SUS activity is highest in carbon sink tissues in plants and low in photosynthetic source tissues, and studies have indicated that SUS is the main sucrose-cleaving activity in sink tissues. Grasses have two isoforms of SUS that are encoded by separate genes. These genes are differentially expressed in different tissues.

Tannin Biosynthetic Pathway

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Condensed tannins are polymerized flavonoids. More specifically, tannins are composed of catechin 4-ol and catechin monomer units, and are stored in the vacuole. In many temperate forage crops, such as ryegrass and fescue, foliar tissues are tannin-negative. This leads to a high initial rate of fermentation when these crops are consumed by ruminant livestock, resulting in both protein degradation and production of ammonia by the livestock. These effects can be reduced by the presence of low to moderate levels of tannin. In certain other plant species, the presence of high levels of tannins reduces palatability and nutritive

value. Introduction of genes encoding enzymes involved in the biosynthesis of condensed tannins into a plant may be employed to synthesize flavonoid compounds that are not normally made in the plant. These compounds may then be isolated and used for treating human or animal disorders or as food additives.

Much of the biosynthetic pathway for condensed tannins is shared with that for anthocyanins, which are pigments responsible for flower color. Therefore, modulation of the levels of enzymes involved in the tannin biosynthetic pathway may be employed to alter the color of foliage and the pigments produced in flowers.

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Most tannins described to date contain pro-cyanidin units derived from dihydroquercetin and pro-delphinidin units derived from dihydromyricetin. However, some tannins contain pro-pelargonidin units derived from dihydrokaempferol. The initial step in the tannin biosynthetic pathway is the condensation of coumaryl CoA with malonyl CoA to give naringenin-chalcone, which is catalyzed by the enzyme chalcone synthase (CHS). The enzyme chalcone isomerase (CHI) catalyzes the isomerization of naringenin chalcone to naringenin (also known as flavanone), which is then hydroxylated by the action of the enzyme flavonone 3- beta-hydroxylase (F3\betaH) to give dihydrokaempferol. The enzyme flavonoid 3'-hydroxylase (F3'OH) catalyzes the conversion of dihydrokaempferol to dihydroquercetin, which in turn can be converted into dihydromyricetin by the action of flavonoid 3'5'-hydroxylase (F3'5'OH). The enzyme dihydroflavonol-4-reductase (DFR) catalyzes the last step before dihydrokaempferol, dihydroquercetin and dihydromyricetin are committed for either anthocyanin (flower pigment) or proanthocyanidin (condensed tannin) formation. DFR also converts dihydrokaempferol to afzelchin-4-ol, dihydroguercetin to catechin-4-ol, and dihydromyricetin to gallocatechin-4-ol, probably by the action of more than one isoform. For a review of the tannin biosynthetic pathway, see, Robbins M.P. and Morris P. in Metabolic Engineering of Plant Secondary Metabolism, Verpoorte and Alfermann (eds), Kluwer Academic Publishers, the Netherlands, 2000.

While polynucleotides encoding some of the enzymes involved in the lignin, fructan and tannin biosynthetic pathways have been isolated for certain species of plants, genes encoding many of the enzymes in a wide range of plant species have not yet been identified.

Thus there remains a need in the art for materials useful in the modification of lignin, fructan and tannin content and composition in plants, and for methods for their use.

Summary of the Invention

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The present invention provides enzymes involved in the lignin, fructan or tannin biosynthetic pathways that are encoded by polynucleotides isolated from forage grass tissues. The polynucleotides were isolated from *Lolium perenne* (perennial ryegrass) and *Festuca arundinacea* (tall fescue) tissues taken at different times of the year, specifically in winter and spring, and from different parts of the plants, including: leaf blades, leaf base, pseudostems, floral stems, roots, inflorescences and stems. The present invention also provides genetic constructs, expression vectors and host cells comprising the inventive polynucleotides, and methods for using the inventive polynucleotides and genetic constructs to modulate the biosynthesis of lignins, fructans and tannins.

In specific embodiments, the isolated polynucleotides of the present invention comprise a sequence selected from the group consisting of: (a) SEQ ID NO: 1-62 and 125-162; (b) complements of SEQ ID NO: 1-62 and 125-162; (c) reverse complements of SEQ ID NO: 1-62 and 125-162; (d) reverse sequences of SEQ ID NO: 1-62 and 125-162; (e) sequences having a 99% probability of being functionally or evolutionarily related to a sequence of (a)-(d), determined as described below; and (f) sequences having at least 75%, 80%, 90% or 98% identity to a sequence of (a)-(d), the percentage identity being determined as described below. Polynucleotides comprising at least a specified number of contiguous residues ("x-mers") of any of SEQ ID NO: 1-62 and 125-162; and oligonucleotide probes and primers corresponding to SEQ ID NO: 1-62 and 125-162 are also provided. All of the above polynucleotides are referred to herein as "polynucleotides of the present invention."

In further aspects, the present invention provides isolated polypeptides comprising an amino acid sequence of SEQ ID NO: 63-124 and 163-190, together with polypeptides comprising a sequence having at least 75%, 80%, 90% or 98% identity to a sequence of SEQ ID NO: 63-124 and 163-190, wherein the polypeptide possesses the same functional activity as the polypeptide comprising a sequence of SEQ ID NO: 63-124 and 163-190. The present invention also contemplates isolated polypeptides comprising at least a functional portion of a polypeptide comprising an amino acid sequence selected from the group consisting of: (a)

SEQ ID NO: 63-124 and 163-190; and (b) sequences having at least 75%, 80%, 90% or 98% identity to a sequence of SEQ ID NO: 63-124 and 163-190.

In another aspect, the present invention provides genetic constructs comprising a polynucleotide of the present invention, either alone, in combination with one or more of the inventive sequences, or in combination with one or more known polynucleotides.

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In certain embodiments, the present invention provides genetic constructs comprising, in the 5'-3' direction: a gene promoter sequence; an open reading frame coding for at least a functional portion of a polypeptide of the present invention; and a gene termination sequence. An open reading frame may be orientated in either a sense or anti-sense direction. Genetic constructs comprising a non-coding region of a polynucleotide of the present invention or a polynucleotide sequence complementary to a non-coding region, together with a gene promoter sequence and a gene termination sequence, are also provided. Preferably, the gene promoter and termination sequences are functional in a host cell, such as a plant cell. Most preferably, the gene promoter and termination sequences are those of the original enzyme genes but others generally used in the art, such as the Cauliflower Mosaic Virus (CMV) promoter, with or without enhancers, such as the Kozak sequence or Omega enhancer, and Agrobacterium tumefaciens nopalin synthase terminator may be usefully employed in the present invention. Tissue-specific promoters may be employed in order to target expression to one or more desired tissues. The construct may further include a marker for the identification of transformed cells.

In a further aspect, transgenic cells, such as transgenic plant cells, comprising the constructs of the present invention are provided, together with tissues and plants comprising such transgenic cells, and fruits, seeds and other products, derivatives, or progeny of such plants.

In yet another aspect, methods for modulating the lignin, fructan or tannin content and composition of a target organism, such as a plant, are provided, such methods including stably incorporating into the genome of the target plant a genetic construct comprising a polynucleotide of the present invention. In a preferred embodiment, the target plant is a forage grass, preferably selected from the group consisting of *Lolium* and *Festuca* species, and most preferably from the group consisting of *Lolium perenne* and *Festuca arundinacea*. In a related aspect, a method for producing a plant having altered lignin, fructan or tannin

composition is provided, the method comprising transforming a plant cell with a genetic construct comprising of the present invention to provide a transgenic cell, and cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth.

In yet a further aspect, the present invention provides methods for modifying the activity of an enzyme in a target organism, such as a plant, comprising stably incorporating into the genome of the target organism a genetic construct of the present invention. In a preferred embodiment, the target plant is a forage grass, preferably selected from the group consisting of *Lolium* and *Festuca* species, and most preferably from the group consisting of *Lolium perenne* and *Festuca arundinacea*.

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Brief Description of the Drawings

Fig. 1 shows the activity of recombinant LpSPP (SEQ ID NO: 8) and FaSPP (SEQ ID NO 7) on dephosphorylating Suc-6-P and Fru-6-P. The pET41a extract was the vector control.

Fig. 2 shows the peroxidase activity of PER3 (SEQ ID NO: 50) and PER5 (SEQ ID NO: 52) as determined by oxidation of ABTS. Horseradish peroxidase of known activity (Sigma, St Louis, MI) was used as a positive control and boiled samples as a negative control.

Fig. 3 shows PCR verification of transgenic *N. benthamiana* plants transformed with Lp6-SFT1 (SEQ ID NO: 3). Genomic DNA was isolated from kanamycin resistant T2 *N. benthamiana* plants and the Lp6-SFT fragment was amplified using specific PCR primers.

Fig. 4 shows PCR verification of transgenic *N. benthamiana* plants transformed with Lp1-SST (SEQ ID NO: 1). Genomic DNA was isolated from kanamycin resistant T2 *N. benthamiana* plants and the Lp1-SST fragment was amplified using specific PCR primers. Plant number 5 is a non-transgenic control.

Fig. 5 shows the fructan level in transgenic N. benthamiana lines transformed with Lp6-SFT1 (SEQ ID NO: 3) and Lp1-SST (SEQ ID NO: 1).

Fig. 6 shows the sucrose synthesizing activity of FaSPS-N (SEQ ID NO: 9) with and without SPP (SEQ ID NO: 8) in mammalian cell extracts. The non-transfected cells are controls.

Fig. 7 shows the sucrose cleaving activity of FaSUS1 (SEQ ID NO: 13) in mammalian cell extracts.

Fig. 8 shows the invertase activity for vacuolar invertase (SEQ ID NO: 25) and two cell wall invertases (SEQ ID NO: 17 and 19); absence of invertase activity from an empty vector (pPICZalphaA) control is also shown.

Detailed Description of the Invention

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The polypeptides of the present invention, and the polynucleotides encoding the polypeptides, have activity in lignin, fructan and tannin biosynthetic pathways in plants. Using the methods and materials of the present invention, the lignin, fructan and/or tannin content of a plant may be modulated by modulating expression of polynucleotides of the present invention, or by modifying the polynucleotides or polypeptides encoded by polynucleotides. The isolated polynucleotides and polypeptides of the present invention may thus be usefully employed in the correction of nutritional imbalances associated with temperate pastures and to increase the yield of animal products from pastures.

The lignin, fructan and/or tannin content of a target organism, such as a plant, may be modified, for example, by incorporating additional copies of genes encoding enzymes involved in the lignin, fructan or tannin biosynthetic pathways into the genome of the target plant. Similarly, a modified lignin, fructan and/or tannin content can be obtained by transforming the target plant with anti-sense copies of such genes. In addition, the number of copies of genes encoding for different enzymes in the lignin, fructan and tannin biosynthetic pathways can be manipulated to modify the relative amount of each monomer unit synthesized, thereby leading to the formation of lignins, fructans or tannins having altered composition.

The present invention thus provides methods for modulating the polynucleotide and/or polypeptide content and composition of an organism, such methods involving stably incorporating into the genome of the organism a genetic construct comprising one or more polynucleotides of the present invention. In one embodiment, the target organism is a plant species, preferably a forage plant, more preferably a grass of the *Lolium* or *Festuca* species, and most preferably *Lolium perenne* or *Festuca arundinacea*. In related aspects, methods for producing a plant having an altered genotype or phenotype is provided, such methods

comprising transforming a plant cell with a genetic construct of the present invention to provide a transgenic cell, and cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth. Plants having an altered genotype or phenotype as a consequence of modulation of the level or content of a polynucleotide or polypeptide of the present invention compared to a wild-type organism, as well as components (seeds, etc.) of such plants, and the progeny of such plants, are contemplated by and encompassed within the present invention.

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The isolated polynucleotides of the present invention have utility in genome mapping, in physical mapping, and in positional cloning of genes. Additionally, the polynucleotide sequences identified as SEQ ID NOS: 1-62 and 125-162 and their variants, may be used to design oligonucleotide probes and primers. Oligonucleotide probes and primers have sequences that are substantially complementary to the polynucleotide of interest over a Oligonucleotide probes designed using the certain portion of the polynucleotide. polynucleotides of the present invention may be employed to detect the presence and examine the expression patterns of genes in any organism having sufficiently similar DNA and RNA sequences in their cells using techniques that are well known in the art, such as slot Oligonucleotide primers designed using the blot DNA hybridization techniques. polynucleotides of the present invention may be used for PCR amplifications. Oligonucleotide probes and primers designed using the polynucleotides of the present invention may also be used in connection with various microarray technologies, including the microarray technology of Affymetrix (Santa Clara, CA).

In a first aspect, the present invention provides isolated polynucleotide sequences identified in the attached Sequence Listing as SEQ ID NO: 1-62 and 125-162, and polypeptide sequences identified in the attached Sequence Listing as SEQ ID NO: 63-124 and 163-190. The polynucleotides and polypeptides of the present invention have demonstrated similarity to the following polypeptides that are known to be involved in lignin, fructan and tannin biosynthetic processes:

TABLE 1

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		
1 and 125	63 and 163	Fructan biosynthesis	Homolog of Sucrose:Sucrose 1-fructosyltransferase (1-SST) isolated from <i>Festuca arundinacea</i> . They contain a typical signature of the glycosyl hydrolases family 32 (amino acid residues 120 to 133). The glycosyl hydrolases family 32 domain signature has a consensus of HYQPxxH/NxxNDPNG, where D is the active site residue (Henrissat, <i>Biochem. J.</i> 280:309-316, 1991).
	64	Fructan biosynthesis	Homolog of Sucrose:Sucrose 1-fructosyltransferase (1-SST) isolated from <i>Festuca arundinacea</i> . It contains a typical signature of the glycosyl hydrolases family 32 (amino acid residues 120 to 133). The glycosyl hydrolases family 32 domain signature has a consensus of HYQPxxH/NxxNDPNG, where D is the active site residue (Henrissat, <i>Biochem. J.</i> 280:309-316, 1991).
3 and 126	65 and 164	Fructan biosynthesis	Homolog of Sucrose:fructan 6-fructosyltransferase (6-SFT) isolated from <i>Festuca arundinacea</i> . They contain a typical signature of the glycosyl hydrolases family 32 (amino acid residues 90 to 564). The glycosyl hydrolases family 32 domain signature has a consensus of HYQPxxH/NxxNDPNG, where D is the active site residue (Henrissat, <i>Biochem. J.</i> 280:309-316, 1991).
4 and 127	66 and 165	Fructan biosynthesis	Homolog of Sucrose: fructan 6-fructosyltransferase (6-SFT) isolated from <i>Lolium</i> perenne. They contain a typical signature of the glycosyl hydrolases family 32 (amino acid residues 96 to 107). The glycosyl hydrolases family 32 domain signature has a consensus of HYQPxxH/NxxNDPNG, where D is the active site residue (Henrissat, <i>Biochem. J.</i> 280:309-316, 1991).
5	67	Fructan biosynthesis	Homolog of sucrose:fructan 6-fructosyltransferase (6-SFT) isolated from <i>Festuca</i> arundinacea.
6 and 128	68 and 166	Fructan biosynthesis	Homolog of Sucrose: fructan 6-fructosyltransferase (6-SFT) isolated from <i>Lolium</i> perenne. They contain a typical signature of the glycosyl hydrolases family 32 (amino acid

	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		
			residues 90 to 103). The glycosyl hydrolases family 32 domain signature has a consensus of HYQPxxH/NxxNDPNG, where D is the active site residue (Henrissat, <i>Biochem. J.</i> 280:309-316, 1991).
7 and 129	69	Fructan biosynthesis	Homolog of Sucrose-6-phosphate phosphohydrolase (SPP; EC 3.1.3.24) isolated from Festuca arundinacea. This enzyme belongs to the superfamily of hydrolases, and has the three conserved motifs found in these proteins (Galperin and Koonin, Trends Biochem Sci. 23:127-129, 1998). Motif I (amino acid residues 10 to 19) contains conserved Asp and a Thr residues, motif II (amino acid residues 48 to 53) contains a conserved Thr residue, and Motif III (residues 167 to 220) contains conserved Lys (position 167) and Asp residues (position 202 and 206). These conserved amino acid residues are required for activity of the enzyme.
8	70	Fructan biosynthesis	Homolog of Sucrose-6-phosphate phosphohydrolase (SPP; EC 3.1.3.24) isolated from Lolium perenne. This enzyme belongs to the superfamily of hydrolases, and has the three conserved motifs found in these proteins (Galperin and Koonin, Trends Biochem Sci. 23:127-129, 1998). Motif I (residues 10 to 19) contains conserved Asp and Thr residues, motif II (amino acid residues 48 to 53) contains a conserved Thr residue, and Motif III (amino acid residues 167 to 220) contains conserved Lys (position 167) and Asp residues (position 202 and 206). These conserved amino acid residues are required for activity of the enzyme.
9 and 130	71	Fructan biosynthesis	Homolog of sucrose phosphate synthase (SPS-1) isolated from <i>Festuca arundinacea</i> .
10 and 131	72 and 167	Fructan biosynthesis	Homolog of sucrose phosphate synthase (SPS-1) isolated from <i>Lolium perenne</i> and that is involved in the sucrose synthesis pathway.
11 and 132	73 and 168	Fructan biosynthesis	Homolog of sucrose phosphate synthase (SPS-N) isolated from <i>Lolium perenne</i> and that is involved in the sucrose synthesis pathway.
12 and 133	74 and 169	Fructan	Homolog of sucrose synthase (SuS) isolated

Polynucleotide Polypeptide biosynthesis from Lolium perenne. These molecules com a leucine zipper motif in amino acid position 191 to 213. Leucine zipper motifs are prese in many gene regulatory proteins (Landschu et al., Science 240:1759-1764, 1988). Fructan biosynthesis from Festuca arundinacea. This molecule contains a leucine zipper motif in amino acid position 191 to 213. Leucine zipper motifs a	
a leucine zipper motif in amino acid position 191 to 213. Leucine zipper motifs are prese in many gene regulatory proteins (Landschu et al., Science 240:1759-1764, 1988). 13 75 Fructan biosynthesis from Festuca arundinacea. This molecule contains a leucine zipper motif in amino acid position 191 to 213. Leucine zipper motifs a	olynucleotide
13 75 Fructan Homolog of sucrose synthase (SuS) isolated biosynthesis from Festuca arundinacea. This molecule contains a leucine zipper motif in amino acid position 191 to 213. Leucine zipper motifs a	
biosynthesis from Festuca arundinacea. This molecule contains a leucine zipper motif in amino acid position 191 to 213. Leucine zipper motifs a	10
present in many gene regulatory proteins (Landschulz et al., Science 240:1759-1764, 1988).	
14 and 134 76 and 170 Fructan Homolog of sucrose synthase (SuS) isolated biosynthesis from Lolium perenne.	14 and 134
15 77 Fructan Homolog of sucrose synthase (SuS) isolated	15
biosynthesis from Festuca arundinacea.	
16 and 135 78 and 171 Fructan biosynthesis Fructan biosynthesis isolated from Festuca arundinacea that below to the family 32 of glycosyl hydrolases. The molecules contain a conserved peptide domain amino acid residues 139 to 144 and 242-2 respectively. The consensus peptide domain invertases is (WVYL)EC(PIL)D (LFI) with conserved Cys residue part of the catalytic domain (Sturm, Plant Physiol. 121:1-7, 199).	
Fructan biosynthesis Homolog of cell wall invertase (CWINV) isolated from Lolium perenne that belongs to the family 32 of glycosyl hydrolases. This molecule contains a conserved pentapeptide bF-motif at amino acid residues 70 to 74 and peptide domain in amino acid residues 250 to 255. The consensus peptide domain of invertases is (WVYL)EC(PIL)D(LFI) with the conserved Cys residue part of the catalytic domain (Sturm, Plant Physiol. 121:1-7, 199). It also contains a glycosyl hydrolases family signature region at amino acids 61 to 74 that contains a conserved His residue important the catalytic reaction (Reddy and Maley, J. Biol. Chem. 265:10817-10120, 1990).	17
18 and 136 80 and 172 Fructan Homolog of cell wall invertase (CWINV)	18 and 136
biosynthesis isolated from <i>Lolium perenne</i> that belongs to the family 32 of glycosyl hydrolases.	
19 81 Fructan Homolog of cell wall invertase (CWINV)	19

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		
			isolated from Festuca arundinacea that belongs to the family 32 of glycosyl hydrolases. This molecule contains a conserved pentapeptide bF-motif at amino acid residues 60 to 64. The consensus peptide domain of invertases is (WVYL)EC(PIL)D(LFI) with the conserved Cys residue part of the catalytic domain (Sturm, Plant Physiol. 121:1-7, 1999). It also contains a glycosyl hydrolases family 32 signature region at amino acids 51 to 64 that contains a conserved His residue important in the catalytic reaction (Reddy and Maley, J. Biol. Chem. 265:10817-10120, 1990). A signal peptide is present in amino acid residues 1 to 24.
20 and 137	82 and 173	Fructan biosynthesis	Homolog of cell wall invertase (CWINV) isolated from <i>Festuca arundinacea</i> that belongs to the family 32 of glycosyl hydrolases. These molecules contain a peptide domain in amino acid residues 61 to 66and 242-247, respectively. The consensus peptide domain of invertases is (WVYL)EC(PIL)D(LFI) with the conserved Cys residue part of the catalytic domain (Sturm, <i>Plant Physiol.</i> 121:1-7, 1999).
21	83	Fructan biosynthesis	Homolog of cell wall invertase (CWINV) isolated from Festuca arundinacea that belongs to the family 32 of glycosyl hydrolases. This molecule contains a conserved pentapeptide bF-motif at amino acid residues 73 to 77 and a peptide domain in amino acid residues 253 to 258. The consensus peptide domain of invertases is (WVYL)EC(PIL)D-(LFI) with the conserved Cys residue part of the catalytic domain (Sturm, Plant Physiol. 121:1-7, 1999). It also contains a glycosyl hydrolases family 32 signature region at amino acid 64 to 77 that contains a conserved His residue important in the catalytic reaction (Reddy and Maley, J. Biol. Chem. 265:10817-10120, 1990).
22 and 138	84 and 174	Fructan biosynthesis	Homolog of cell wall invertase (CWINV) isolated from <i>Lolium perenne</i> that belongs to the family 32 of glycosyl hydrolases. These molecules contain a peptide domain in amino acid residues 174 to 179 and 234 to 239,

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide			
			respectively. The consensus peptide domain of invertases is (WVYL)EC- (PIL)D(LFI) with the conserved Cys residue part of the catalytic domain (Sturm, <i>Plant Physiol</i> . 121:1-7, 1999).
23	85	Fructan biosynthesis	Homolog of cell wall invertase (CWINV) isolated from Festuca arundinacea that belongs to the family 32 of glycosyl hydrolases. This molecule contains a conserved pentapeptide bF-motif at amino acid residues 56 to 60. The consensus peptide domain of invertases is (WVYL)EC(PIL)D(LFI) with the conserved Cys residue part of the catalytic domain (Sturm, Plant Physiol. 121:1-7, 1999). It also contains a glycosyl hydrolases family 32 signature region at amino acid 47 to 60 that contains a conserved His residue that is important in the catalytic reaction (Reddy and Maley, J. Biol. Chem. 265:10817-10120, 1990). A signal peptide is present in amino acid residues 1 to 22.
24 and 139	86 and 175	Fructan biosynthesis	Homolog of cell wall invertase (CWINV) isolated from Lolium perenne that belongs to the family 32 of glycosyl hydrolases. These molecules contain a conserved pentapeptide bF-motif at amino acid residues 244 to 249. The consensus peptide domain of invertases is (WVYL)EC(PIL)D(LFI) with the conserved Cys residue part of the catalytic domain (Sturm, Plant Physiol. 121:1-7, 1999). They also contain a glycosyl hydrolases family 32 signature region at amino acid 56 to 69 that contains a conserved His residue that is important in the catalytic reaction (Reddy and Maley, J. Biol. Chem. 265:10817-10120, 1990). A signal peptide is present in amino acid residues 1 to 25.
25 and 140	87 and 176	Fructan biosynthesis	Homolog of vacuolar invertase (SINV) isolated from <i>Lolium perenne</i> that belongs to the family 32 of glycosyl hydrolases. These molecules contain a conserved pentapeptide bF-motif at amino acid residues 136 to 140 and 138 to 142, respectively and a peptide domain in amino acid residues 317 to 322 and 319 to 324, respectively. The consensus peptide domain of

	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		invertases is (WVYL)EC(PIL)D(LFI) with the
			conserved Cys residue part of the catalytic
			domain (Sturm, Plant Physiol. 121:1-7, 1999).
			It also contains a glycosyl hydrolases family 32
			signature region at amino acid 127 to 140 and
			129 to 142 that contains a conserved His
			residue that is important in the catalytic
			reaction (Reddy and Maley, <i>J. Biol. Chem.</i> 265:10817-10120, 1990).
26 and 141	88 and 177	Fructan	Homolog of invertase (SINV) isolated from
20 and 141	00 and 177	biosynthesis	Lolium perenne that belongs to the family 32 of
		2103J 11012022	glycosyl hydrolases. These molecules contain a
			peptide domain in amino acid residues 143 to
			148 and 184 to 189, respectively. The
			consensus peptide domain of invertases is
			(WVYL)EC(PIL)D(LFI) with the conserved
			Cys residue part of the catalytic domain (Sturm, <i>Plant Physiol</i> . 121:1-7, 1999).
27	89	I ionin/Tannin	Homolog of 4-Coumarate: CoA ligase (4CL,
	67	biosynthesis	EC 6.2.1.12) isolated from <i>Lolium perenne</i> The
		J	molecule has two conserved AMP binding
			regions at amino acid residues 182 to 193 and
			383 to 389 (Hu et al., Proc. Natl. Acad. Sci.
			USA 95:5407-5412, 1998). The AMP-binding
			domain signature consists of two binding site motifs. The consensus of the first motif is
			LPYSSGTTGLPK (Etchegaray et al.,
			Biochem. Mol. Biol. Int. 44:235-243, 1998).
			The region very rich in glycine, serine, and
			threonine followed by a conserved lysine. In
			most of these proteins, the residue that follows
			the Lys at the end of the pattern is a Gly. The
			second motif consensus sequence is GEIC(V/I)RG (Hu et al., Proc. Natl. Acad. Sci.
			USA 95:5407-5412, 1998).
28 and 142	90	Lignin/Tannin	Homolog of 4-Coumarate:CoA ligase (4CL,
20 4.10 2 12		biosynthesis	EC 6.2.1.12) isolated from <i>Lolium perenne</i> .
}			The molecule has two conserved AMP binding
			regions at amino acid residues 195 to 206 and
			395 to 401 (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998). The AMP-binding
			domain signature consists of two binding site
			motifs. The consensus of the first motif is
			LPYSSGTTGLPK (Etchegaray et al.,

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		1,000
			Biochem. Mol. Biol. Int. 44:235-243, 1998). The region very rich in glycine, serine, and threonine followed by a conserved lysine. In most of these proteins, the residue that follows the Lys at the end of the pattern is a Gly. The second motif consensus sequence is GEIC(V/I)RG (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998).
29	91	biosynthesis	Homolog of 4-Coumarate:CoA ligase (4CL, EC 6.2.1.12) isolated from Festuca arundinacea. The molecule has two conserved AMP binding regions at amino acid residues 195 to 206 and 395 to 401 (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998). The AMP-binding domain signature consists of two binding site motifs. The consensus of the first motif is LPYSSGTTGLPK (Etchegaray et al., Biochem. Mol. Biol. Int. 44:235-243, 1998). The region very rich in glycine, serine, and threonine followed by a conserved lysine. In most of these proteins, the residue that follows the Lys at the end of the pattern is a Gly. The second motif consensus sequence is GEIC(V/I)RG (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998).
30 and 143	92 and 178	Lignin/Tannin biosynthesis	Homolog of 4-Coumarate:CoA ligase (4CL, EC 6.2.1.12) isolated from Lolium. The molecules have two conserved AMP binding regions at amino acid residues 194 to 205 and 394 to 400 (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998). The AMP-binding domain signature consists of two binding site motifs. The consensus of the first motif is LPYSSGTTGLPK (Etchegaray et al., Biochem. Mol. Biol. Int. 44:235-243, 1998). The region very rich in glycine, serine, and threonine followed by a conserved lysine. In most of these proteins, the residue that follows the Lys at the end of the pattern is a Gly. The second motif consensus sequence is GEIC(V/I)RG (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998).
31	93		Homolog of 4-Coumarate:CoA ligase (4CL,
	1	biosynthesis	EC 6.2.1.12) isolated from Festuca

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide			
		·	arundinacea. The molecule has two conserved AMP binding regions at amino acid residues 194 to 206 and 482 to 490 (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998). The AMP-binding domain signature consists of two binding site motifs. The consensus of the first motif is LPYSSGTTGLPK (Etchegaray et al., Biochem. Mol. Biol. Int. 44:235-243, 1998). The region very rich in glycine, serine, and threonine followed by a conserved lysine. In most of these proteins, the residue that follows the Lys at the end of the pattern is a Gly. The second motif consensus sequence is GEIC(V/I)RG (Hu et al., Proc. Natl. Acad. Sci. USA 95:5407-5412, 1998).
32 and 144	94 and 179	biosynthesis	Homolog of cinnamic acid 4-hydroxylase (C4H) isolated from <i>Lolium perenne</i> . The molecules have a conserved cytochrome P450 region in amino acids 436 to 445 that contains a conserved Cys residue involved in heme binding (Miles <i>et al.</i> , <i>Biochim Biophys Acta</i> 1543:383-407, 2000).
33	95	Lignin/Tannin biosynthesis	Homolog of cinnamic acid 4-hydroxylase (C4H) isolated from Festuca arundinacea. The molecule has a conserved Cytochrome P450 region in amino acids 440 to 449 that contains a conserved Cys residue involved in heme binding. The cytochrome P450 cysteine hemeiron ligand signature consensus is FGxGRRSCPG where the conserved C is the heme iron ligand (Miles et al., Biochim. Biophys. Acta 1543:383-407, 2000). It also contains an aldehyde dehydrogenases active site (Hempel et al., Adv. Exp. Med. Biol. 436:53-59, 1999) at amino acid residues 428 to 435. A hydrophobic signal peptide region is present in amino acid residues 1 to 24.
34 and 145	96 and 180	Lignin biosynthesis	Homolog of cinnamyl-alcohol dehydrogenase (CAD; EC 1.1.1.195) isolated from <i>Lolium</i> perenne. These molecules contain a conserved zinc-containing alcohol dehydrogenase domain (Joernvall et al., Eur. J. Biochem. 167:195-201, 1987) in amino acid residues 69 to 83, with a conserved His residue at position 70. They also

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		
			contain a cytochrome C family heme-binding site signature (Mathews, <i>Prog. Biophys. Mol. Biol.</i> 45:1-56, 1985) in residues 45 to 50.
35	97	Lignin biosynthesis	Homolog of cinnamyl-alcohol dehydrogenase (CAD; EC 1.1.1.195) isolated from Festuca arundinacea. CAD belongs to the family of zinc-binding dehydrogenases. This molecule contains a conserved zinc-containing alcohol dehydrogenases domain (Joernvall et al., Eur. J. Biochem. 167:195-201, 1987) in amino acid residues 69 to 83, with a conserved His residue at position 70. It also contains a Cytochrome C family heme-binding site signature. The cytochrome C family heme-binding site signature is CGICHT. In the cytochrome C protein family, the heme group is covalently attached by thioether bonds to two conserved cysteine residues. The consensus sequence for this site is Cys-X-X-Cys-His and the histidine residue is one of the two axial ligands of the heme iron. This arrangement is shared by all proteins known to belong to cytochrome C family (Mathews, Prog. Biophys. Mol. Biol. 45:1-56, 1985).
36 and 146	98	Lignin biosynthesis	Homolog of caffeoyl coenzyme A O-methyltransferase (CCoAOMT) (EC 2.1.1.104) isolated from <i>Lolium perenne</i> .
. 37	99	Lignin biosynthesis	Homolog of caffeoyl coenzyme A O-methyltransferase (CCoAOMT) (EC 2.1.1.104) isolated from <i>Festuca arundinacea</i> .
38 and 147	100 and 181	Lignin biosynthesis	Homolog of cinnamoyl CoA:NADP oxidoreductase (CCR, EC 1.2.1.44) isolated from <i>Lolium perenne</i> that catalyzes the conversion of cinnamoyl CoA esters to their corresponding cinnamaldehydes in the first specific step in the synthesis of the lignin monomers. A hydrophobic region typical of a signal peptide is present in amino acid residues 1 to 24.
39 and 148	101	Lignin biosynthesis	Homolog of cinnamoyl CoA:NADP oxidoreductase (CCR, EC 1.2.1.44) isolated from <i>Festuca arundinacea</i> that catalyzes the conversion of cinnamoyl CoA esters to their corresponding cinnamaldehydes in the first

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		
			specific step in the synthesis of the lignin
			monomers.
40 and 149	102 and 182	Lignin	Homolog of caffeic acid 3-O-methyltransferase
		biosynthesis	(COMT1) isolated from Festuca arundinacea
			A conserved consensus phosphopantetheine attachment site was identified in amino acid
			residues 47 to 62. This domain is involved in
			the attachment of activated fatty acid and
			amino-acid groups, with the Ser residue at
			position 52 crucial for the phosphopantetheine
			attachment (Pugh and Wakil, J. Biol. Chem.
			240:4727-4733, 1965).
41 and 150	103	Lignin	Homolog of caffeic acid 3-O-methyltransferase
11 4111 10 1		biosynthesis	(COMT1) isolated from Lolium perenne A
		·	conserved consensus phosphopantetheine
			attachment site was identified in amino acid
			residues 47 to 62. This domain is involved in
			the attachment of activated fatty acid and
			amino-acid groups, with the Ser residue at
			position 52 crucial for the phosphopantetheine
			attachment (Pugh and Wakil, J. Biol. Chem.
40	104	Т::-	240:4727-4733, 1965). Homolog of caffeic acid 3-O-methyltransferase
42	104	Lignin biosynthesis	(COMT1) isolated from Festuca arundinacea
		olosyllulesis	A conserved consensus phosphopantetheine
			attachment site was identified in amino acid
			residues 47 to 62. This domain is involved in
			the attachment of activated fatty acid and
			amino-acid groups, with the Ser residue at
			position 52 crucial for the phosphopantetheine
			attachment (Pugh and Wakil, J. Biol. Chem.
			240:4727-4733, 1965).
43	105	Lignin	Homolog of caffeic acid 3-O-methyltransferase
		biosynthesis	(COMT1) isolated from Lolium perenne A
			conserved consensus phosphopantetheine attachment site was identified in amino acid
			residues 47 to 62. This domain is involved in
·			the attachment of activated fatty acid and
			amino-acid groups, with the Ser residue at
			position 52 crucial for the phosphopantetheine
			attachment (Pugh and Wakil, J. Biol. Chem.
			240:4727-4733, 1965).
44 and 151	106 and 183	Lignin	Homolog of ferulate 5-hydroxylase (F5H)
		biosynthesis	isolated from Lolium perenne. The molecules

	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
			have a conserved cytochrome P450 region in
			amino acids 463 to 472 that contains a
			conserved Cys residue involved in heme
			binding (Miles et al., Biochim Biophys Acta
			1543:383-407, 2000). A signal peptide is present in amino acid residues 1 to 30.
45	107	T::	Homolog of ferulate 5-hydroxylase (F5H)
45	107		isolated from Festuca arundinaceae. The
		Diosymmesis	molecule has a conserved cytochrome P450
			region in amino acids 462 to 471 that contains
			a conserved Cys residue involved in heme
			binding (Miles et al., Biochim Biophys Acta
			1543:383-407, 2000). A signal peptide is
			present in amino acid residues 1 to 30.
46 and 152	108	Lignin/Tannin	Homolog of phenylalanine ammonia-lyase (EC
		biosynthesis	4.3.1.5) (PAL) isolated from <i>Lolium perenne</i> .
			The polypeptide has a conserved PAL-histidase
			region in amino acid residues 193 to 209.
47 and 153	109 and 184		Homolog of phenylalanine ammonia-lyase (EC
		biosynthesis	4.3.1.5) (PAL) isolated from Festuca
			arundinacea. A conserved phenylalanine and
			histidine ammonia-lyases active site signature has been identified in amino acid residues 195
			to 210.
48	110	Lignin	Homolog of peroxidase (PER) isolated from
46	110	biosynthesis	Festuca arundinacea. The conserved
		Crosynatesis	peroxidase I region is present in amino acid
			residues 188 to 199 and contains a conserved
			His residue at position 196 in the active site,
			and the conserved peroxidase 2 region is
			present in amino acid residues 60 to 71, with a
			conserved His residue at position 69 that is
			involved in heme binding (Kimura and Ikeda-
			Saito, Proteins 3:113-120, 1988). A signal
			peptide is present in amino acid residues 1 to 27.
49	111	Lignin	Homolog of peroxidase (PER) isolated from
		biosynthesis	Lolium perenne. The conserved peroxidase I
			region is present in amino acid residues 199 to
			209 and contains a conserved His residue at
			position 208 in the active site. A signal peptide
			is present in amino acid residues 1 to 33.
50	112	Lignin	Homolog of peroxidase (PER) isolated from
		biosynthesis	Festuca arundinacea. The conserved

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		
			peroxidase I region is present in amino acid residues 180 to 190 and contains a conserved His residue at position 188 in the active site, and the conserved peroxidase 2 region is present in amino acid residues 55 to 66, with a conserved His residue at position 64 that is involved in heme binding (Kimura and Ikeda-Saito, <i>Proteins</i> 3:113-120, 1988). A signal peptide is present in amino acid residues 1 to 22.
51 and 154	113	Lignin biosynthesis	Homolog of peroxidase (PER) isolated from Lolium perenne. The conserved peroxidase I region is present in amino acid residues 199 to 209 and contains a conserved His residue at position 207 in the active site, and the conserved peroxidase 2 region is present in amino acid residues 70 to 80, with a conserved His residue at position 78 that is involved in heme binding (Kimura and Ikeda-Saito, Proteins 3:113-120, 1988). A signal peptide is present in amino acid residues 1 to 20.
52 and 155	114	Lignin biosynthesis	Homolog of peroxidase (PER) isolated from <i>Lolium perenne</i> . The conserved peroxidase I region is present in amino acid residues 198 to 208 and contains a conserved His residue at position 206 in the active site (Kimura and Ikeda-Saito, <i>Proteins</i> 3:113-120, 1988). A signal peptide is present in amino acid residues 1 to 34.
53, 156, and 162	115, 185, and 190	Lignin biosynthesis	Homolog of peroxidase (PER) isolated from <i>Lolium perenne</i> . The conserved peroxidase I region is present in amino acid residues 157 to 168, 188 to 199, and 190 to 201, respectively and contain a conserved His residue at position 165, 196 and 198, respectively in the active site, and the conserved peroxidase 2 region is present in amino acid residues 29 to 41, 60 to 72 and 62 to 74, respectively, with a conserved His residue at position 38, 69 and 71, respectively that is involved in heme binding (Kimura and Ikeda-Saito, <i>Proteins</i> 3:113-120, 1988).
54	116	Lignin biosynthesis	Homolog of peroxidase (PER) isolated from Festuca arundinacea. The conserved

SEQ ID NO	SEQ ID NO	Category	Description
Polynucleotide	Polypeptide		
			peroxidase I region is present in amino acid residues 176 to 186 and contains a conserved His residue at position 184 in the active site, and the conserved peroxidase 2 region is present in amino acid residues 55 to 67, with a conserved His residue at position 64 that is involved in heme binding (Kimura and Ikeda-Saito, <i>Proteins</i> 3:113-120, 1988). A signal peptide is present in amino acid residues 1 to 22.
55	117	Tannin Biosynthesis	Homolog of chalcone isomerase (CHI) isolated from <i>Lolium perenne</i> . The molecule contains a chalcone isomerase region at amino acid residues 1 to 213.
56	118	Tannin Biosynthesis	Homolog of chalcone isomerase (CHI). The molecule contains a chalcone isomerase region at amino acid residues 23 to 235.
57 and 157	119 and 186	Tannin Biosynthesis	Homolog of Chalcone Synthase (CHS) isolated from <i>Lolium perenne</i> and that is an important enzyme in flavonoid synthesis. The molecules contain a conserved chalcone synthase active site (Lanz <i>et al.</i> , <i>J. Biol. Chem.</i> 266:9971-9976, 1991) at amino acid residues 166 to 175, with the conserved Cys residue at position 167.
58 and 158	120 and 187	Tannin Biosynthesis	Homolog of dihydroflavonal-4-reductase (DFR) isolated from <i>Festuca arundinacea</i> .
59 and 159	121 and 188	Tannin Biosynthesis	Homolog of dihydroflavonal-4-reductase (DFR) isolated from <i>Lolium perenne</i> .
60 and 160	122 and 189	Tannin Biosynthesis	Homolog of dihydroflavonal-4-reductase (DFR) isolated from <i>Lolium perenne</i> . These molecules contain a conserved ATP/GTP binding site at amino acid residues 84 to 91 and 86 to 93, respectively, known as the "A" sequence (Walker <i>et al.</i> , <i>EMBO J.</i> 1:945-951, 1982) or "P-loop" (Saraste <i>et al.</i> , <i>Trends Biochem. Sci.</i> 15:430-434, 1990).
61 and 161	123	Tannin biosynthesis	Homolog of flavanone 3- β hydroxylase (F3 β H) isolated from <i>Lolium perenne</i> .
62	124	Tannin Biosynthesis	Homolog of flavanone 3- β hydroxylase (F3 β H) isolated from <i>Festuca arundinacea</i> .

All the polynucleotides and polypeptides provided by the present invention are isolated and purified, as those terms are commonly used in the art. Preferably, the polypeptides and polynucleotides are at least about 80% pure, more preferably at least about 90% pure, and most preferably at least about 99% pure.

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The word "polynucleotide(s)," as used herein, means a polymeric collection of nucleotides, and includes DNA and corresponding RNA molecules and both single and double stranded molecules, including RNAi, HnRNA and mRNA molecules, sense and antisense strands of DNA and RNA molecules, and comprehends cDNA, genomic DNA, and wholly or partially synthesized polynucleotides. A polynucleotide of the present invention may be an entire gene, or any portion thereof. As used herein, a "gene" is a DNA sequence which codes for a functional protein or RNA molecule. Operable anti-sense polynucleotides may comprise a fragment of the corresponding polynucleotide, and the definition of "polynucleotide" therefore includes all operable anti-sense fragments. Anti-sense polynucleotides and techniques involving anti-sense polynucleotides are well known in the art and are described, for example, in Robinson-Benion *et al.*, *Methods in Enzymol*. 254(23): 363-375, 1995 and Kawasaki *et al.*, *Artific. Organs* 20(8): 836-848, 1996.

In specific embodiments, the present invention provides isolated polynucleotides comprising a sequence of SEQ ID NO: 1-62 and 125-162; polynucleotides comprising variants of SEQ ID NO: 1-62 and 125-162; polynucleotides comprising extended sequences of SEQ ID NO: 1-62 and 125-162 and their variants, oligonucleotide primers and probes corresponding to the sequences set out in SEQ ID NO: 1-62 and 125-162 and their variants, polynucleotides comprising at least a specified number of contiguous residues of any of SEQ ID NO: 1-62 and 125-162 (x-mers), and polynucleotides comprising extended sequences which include portions of the sequences set out in SEQ ID NO: 1-62 and 125-162, all of which are referred to herein, collectively, as "polynucleotides of the present invention." Polynucleotides that comprise complements of such polynucleotide sequences, reverse complements of such polynucleotide sequences, together with variants of such sequences, are also provided.

The definition of the terms "complement(s)," "reverse complement(s)," and "reverse sequence(s)," as used herein, is best illustrated by the following example. For the sequence 5' AGGACC 3', the complement, reverse complement, and reverse sequence are as follows:

complement

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3' TCCTGG 5'

reverse complement

3' GGTCCT 5'

reverse sequence

5' CCAGGA 3'.

Preferably, sequences that are complements of a specifically recited polynucleotide sequence are complementary over the entire length of the specific polynucleotide sequence.

As used herein, the term "x-mer," with reference to a specific value of "x," refers to a polynucleotide comprising at least a specified number ("x") of contiguous residues of: any of the polynucleotides provided in SEQ ID NO: 1-62 and 125-162. The value of x may be from about 20 to about 600, depending upon the specific sequence.

Polynucleotides of the present invention comprehend polynucleotides comprising at least a specified number of contiguous residues (x-mers) of any of the polynucleotides identified as SEQ ID NO: 1-62 and 125-162, or their variants. Similarly, polypeptides of the present invention comprehend polypeptides comprising at least a specified number of contiguous residues (x-mers) of any of the polypeptides identified as SEQ ID NO: 63-124 and 163-190. According to preferred embodiments, the value of x is at least 20, more preferably at least 40, more preferably yet at least 60, and most preferably at least 80. Thus, polynucleotides of the present invention include polynucleotides comprising a 20-mer, a 40mer, a 60-mer, an 80-mer, a 100-mer, a 120-mer, a 150-mer, a 180-mer, a 220-mer, a 250mer; or a 300-mer, 400-mer, 500-mer or 600-mer of a polynucleotide provided in SEQ ID NO: 1-62 and 125-162, or a variant of one of the polynucleotides corresponding to the polynucleotides provided in SEQ ID NO: 1-62 and 125-162. Polypeptides of the present invention include polypeptides comprising a 20-mer, a 40-mer, a 60-mer, an 80-mer, a 100mer, a 120-mer, a 150-mer, a 180-mer, a 220-mer, a 250-mer; or a 300-mer, 400-mer, 500mer or 600-mer of a polypeptide provided in SEQ ID NO: 63-124 and 163-190, or a variant thereof.

Polynucleotides of the present invention were isolated by high throughput sequencing of cDNA libraries comprising forage grass tissue collected from *Lolium perenne* and *Festuca arundinacea*. Some of the polynucleotides of the present invention may be "partial" sequences, in that they do not represent a full-length gene encoding a full-length polypeptide. Such partial sequences may be extended by analyzing and sequencing various DNA libraries using primers and/or probes and well known hybridization and/or PCR techniques. Partial

sequences may be extended until an open reading frame encoding a polypeptide, a full-length polynucleotide and/or gene capable of expressing a polypeptide, or another useful portion of the genome is identified. Such extended sequences, including full-length polynucleotides and genes, are described as "corresponding to" a sequence identified as one of the sequences of SEQ ID NO: 1-62 and 125-162 or a variant thereof, or a portion of one of the sequences of SEQ ID NO: 1-62 and 125-162 or a variant thereof, when the extended polynucleotide comprises an identified sequence or its variant, or an identified contiguous portion (x-mer) of one of the sequences of SEQ ID NOS: 1-62 and 125-162 or a variant thereof. Similarly, RNA sequences, reverse sequences, complementary sequences, anti-sense sequences and the like, corresponding to the polynucleotides of the present invention, may be routinely ascertained and obtained using the cDNA sequences identified as SEQ ID NOS: 1-62 and 125-162.

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The polynucleotides identified as SEQ ID NOS: 1-62 and 125-162 contain open reading frames ("ORFs") or partial open reading frames encoding polypeptides and Additionally, open reading frames encoding functional portions of polypeptides. polypeptides may be identified in extended or full length sequences corresponding to the sequences set out as SEQ ID NOS: 1-62 and 125-162. Open reading frames may be identified using techniques that are well known in the art. These techniques include, for example, analysis for the location of known start and stop codons, most likely reading frame identification based on codon frequencies, etc. These techniques include, for example, analysis for the location of known start and stop codons, most likely reading frame identification based on codon frequencies, etc. Suitable tools and software for ORF analysis are well known in the art and include, for example, GeneWise, available from The Sanger Center, Wellcome Trust Genome Campus, Hinxton, Cambridge, CB10 1SA, United Kingdom; Diogenes, available from Computational Biology Centers, University of Minnesota, Academic Health Center, UMHG Box 43 Minneapolis MN 55455; and GRAIL, available from the Informatics Group, Oak Ridge National Laboratories, Oak Ridge, Tennessee TN. Once a partial open reading frame is identified, the polynucleotide may be extended in the area of the partial open reading frame using techniques that are well known in the art until the polynucleotide for the full open reading frame is identified.

Once open reading frames are identified in the polynucleotides of the present invention, the open reading frames may be isolated and/or synthesized. Expressible genetic constructs comprising the open reading frames and suitable promoters, initiators, terminators, etc., which are well known in the art, may then be constructed. Such genetic constructs may be introduced into a host cell to express the polypeptide encoded by the open reading frame. Suitable host cells may include various prokaryotic and eukaryotic cells, including plant cells, mammalian cells, bacterial cells, algae and the like.

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The polynucleotides of the present invention may be isolated by high throughput sequencing of cDNA libraries prepared from forage grass tissue, as described below in Example 1. Alternatively, oligonucleotide probes and primers based on the sequences provided in SEQ ID NOS: 1-62 and 125-162 can be synthesized as detailed below, and used to identify positive clones in either cDNA or genomic DNA libraries from forage grass tissue cells by means of hybridization or polymerase chain reaction (PCR) techniques. Hybridization and PCR techniques suitable for use with such oligonucleotide probes are well known in the art (see, for example, Mullis et al., Cold Spring Harbor Symp. Quant. Biol., 51:263, 1987; Erlich, ed., PCR technology, Stockton Press: NY, 1989; and Sambrook et al., eds., Molecular cloning: a laboratory manual, 2nd ed., CSHL Press: Cold Spring Harbor, NY, 1989). In addition to DNA-DNA hybridization, DNA-RNA or RNA-RNA hybridization assays are also possible. In the first case, the mRNA from expressed genes would then be detected instead of genomic DNA or cDNA derived from mRNA of the sample. In the second case, RNA probes could be used. Artificial analogs of DNA hybridizing specifically to target sequences could also be employed. Positive clones may be analyzed by restriction enzyme digestion, DNA sequencing or the like.

The polynucleotides of the present invention may also, or alternatively, be synthesized using techniques that are well known in the art. The polynucleotides may be synthesized, for example, using automated oligonucleotide synthesizers (e.g., Beckman Oligo 1000M DNA Synthesizer; Beckman Coulter Ltd., Fullerton, CA) to obtain polynucleotide segments of up to 50 or more nucleic acids. A plurality of such polynucleotide segments may then be ligated using standard DNA manipulation techniques that are well known in the art of molecular biology. One conventional and exemplary polynucleotide synthesis technique involves synthesis of a single stranded polynucleotide segment having, for

example, 80 nucleic acids, and hybridizing that segment to a synthesized complementary 85 nucleic acid segment to produce a 5 nucleotide overhang. The next segment may then be synthesized in a similar fashion, with a 5 nucleotide overhang on the opposite strand. The "sticky" ends ensure proper ligation when the two portions are hybridized. In this way, a complete polynucleotide of the present invention may be synthesized entirely *in vitro*.

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Oligonucleotide probes and primers complementary to and/or corresponding to SEQ ID NOS: 1-62 and 125-162, and variants of those sequences, are also comprehended by the present invention. Such oligonucleotide probes and primers are substantially complementary to the polynucleotide of interest over a certain portion of the polynucleotide. An oligonucleotide probe or primer is described as "corresponding to" a polynucleotide of the present invention, including one of the sequences set out as SEQ ID NOS: 1-62 and 125-162 or a variant thereof, if the oligonucleotide probe or primer, or its complement, is contained within one of the sequences set out as SEQ ID NOS: 1-62 and 125-162 or a variant of one of the specified sequences.

Two single stranded sequences are said to be substantially complementary when the nucleotides of one strand, optimally aligned and compared, with the appropriate nucleotide insertions and/or deletions, pair with at least 80%, preferably at least 90% to 95%, and more preferably at least 98% to 100%, of the nucleotides of the other strand. Alternatively, substantial complementarity exists when a first DNA strand will selectively hybridize to a second DNA strand under stringent hybridization conditions.

In specific embodiments, the oligonucleotide probes and/or primers comprise at least about 6 contiguous residues, more preferably at least about 10 contiguous residues, and most preferably at least about 20 contiguous residues complementary to a polynucleotide sequence of the present invention. Probes and primers of the present invention may be from about 8 to 100 base pairs in length, preferably from about 10 to 50 base pairs in length, and more preferably from about 15 to 40 base pairs in length. The probes can be easily selected using procedures well known in the art, taking into account DNA-DNA hybridization stringencies, annealing and melting temperatures, potential for formation of loops, and other factors which are well known in the art. Preferred techniques for designing PCR primers are disclosed in Dieffenbach and Dyksler, *PCR Primer: a laboratory manual*, CSHL Press: Cold Spring Harbor, NY, 1995. A software program suitable for designing probes, and especially for

designing PCR primers, is available from Premier Biosoft International, 3786 Corina Way, Palo Alto, CA 94303-4504.

The isolated polynucleotides of the present invention also have utility in genome mapping, in physical mapping, and in positional cloning of genes.

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The polynucleotides identified as SEQ ID NOS: 1-62 and 125-162 were isolated from cDNA clones and represent sequences that are expressed in the tissue from which the cDNA was prepared. RNA sequences, reverse sequences, complementary sequences, anti-sense sequences, and the like, corresponding to the polynucleotides of the present invention, may be routinely ascertained and obtained using the cDNA sequences identified as SEQ ID NOS: 1-62 and 125-162.

Identification of genomic DNA and heterologous species DNA can be accomplished by standard DNA/DNA hybridization techniques, under appropriately stringent conditions, using all or part of a polynucleotide sequence as a probe to screen an appropriate library. Alternatively, PCR techniques using oligonucleotide primers that are designed based on known genomic DNA, cDNA and protein sequences can be used to amplify and identify genomic and cDNA sequences.

In another aspect, the present invention provides isolated polypeptides encoded by the above polynucleotides. As used herein, the term "polypeptide" encompasses amino acid chains of any length, including full-length proteins, wherein the amino acid residues are linked by covalent peptide bonds. The term "polypeptide encoded by a polynucleotide" as used herein, includes polypeptides encoded by a polynucleotide that comprises a partial isolated polynucleotide sequence provided herein. In specific embodiments, the inventive polypeptides comprise an amino acid sequence selected from the group consisting of SEQ ID NO: 63-124 and 163-190, as well as variants of such sequences.

As noted above, polypeptides of the present invention may be produced recombinantly by inserting a polynucleotide sequence of the present invention encoding the polypeptide into an expression vector and expressing the polypeptide in an appropriate host. Any of a variety of expression vectors known to those of ordinary skill in the art may be employed. Expression may be achieved in any appropriate host cell that has been transformed or transfected with an expression vector containing a polynucleotide molecule that encodes a recombinant polypeptide. Suitable host cells include prokaryotes, yeast, and

higher eukaryotic cells. Preferably, the host cells employed are plant, *E. coli*, insect, yeast, or a mammalian cell line such as COS or293T. The polynucleotide sequences expressed in this manner may encode naturally occurring polypeptides, portions of naturally occurring polypeptides, or other variants thereof. The expressed polypeptides may be used in various assays known in the art to determine their biological activity. Such polypeptides may also be used to raise antibodies, to isolate corresponding interacting proteins or other compounds, and to quantitatively determine levels of interacting proteins or other compounds.

In a related aspect, polypeptides are provided that comprise at least a functional portion of a polypeptide having an amino acid sequence selected from the group consisting of sequences provided in SEQ ID NO: 63-124 and 163-190, and variants thereof. As used herein, the "functional portion" of a polypeptide is that portion which contains an active site essential for affecting the function of the polypeptide, for example, a portion of the molecule that is capable of binding one or more reactants. The active site may be made up of separate portions present on one or more polypeptide chains and will generally exhibit high binding affinity. Functional portions of a polypeptide may be identified by first preparing fragments of the polypeptide by either chemical or enzymatic digestion of the polypeptide, or by mutation analysis of the polynucleotide that encodes the polypeptide and subsequent expression of the resulting mutant polypeptides. The polypeptide fragments or mutant polypeptides are then tested to determine which portions retain biological activity, using methods well known to those of skill in the art, including the representative assays described below.

Portions and other variants of the inventive polypeptides may be generated by synthetic or recombinant means. Synthetic polypeptides having fewer than about 100 amino acids, and generally fewer than about 50 amino acids, may be generated using techniques well known to those of ordinary skill in the art. For example, such polypeptides may be synthesized using any of the commercially available solid-phase techniques, such as the Merrifield solid-phase synthesis method, where amino acids are sequentially added to a growing amino acid chain. See Merrifield, J. Am. Chem. Soc. 85:2149-2146, 1963. Equipment for automated synthesis of polypeptides is commercially available from suppliers such as Perkin Elmer/Applied Biosystems, Inc. (Foster City, California), and may be operated according to the manufacturer's instructions. Variants of a native polypeptide may

be prepared using standard mutagenesis techniques, such as oligonucleotide-directed site-specific mutagenesis (Kunkel, *Proc. Natl. Acad. Sci. USA* 82:488-492, 1985). Sections of DNA sequences may also be removed using standard techniques to permit preparation of truncated polypeptides.

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As used herein, the term "variant" comprehends nucleotide or amino acid sequences different from the specifically identified sequences, wherein one or more nucleotides or amino acid residues is deleted, substituted, or added. Variants may be naturally occurring allelic variants, or non-naturally occurring variants. Variant sequences (polynucleotide or polypeptide) preferably exhibit at least 75%, more preferably at least 80%, more preferably at least 90%, more preferably yet at least 95% and most preferably, at least 98% identity to a sequence of the present invention. The percentage identity is determined by aligning the two sequences to be compared as described below, determining the number of identical residues in the aligned portion, dividing that number by the total number of residues in the inventive (queried) sequence, and multiplying the result by 100.

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Polynucleotides and polypeptides having a specified percentage identity to a polynucleotide or polypeptide identified in one of SEQ ID NO: 1-190 thus share a high degree of similarity in their primary structure. In addition to a specified percentage identity to a polynucleotide or polypeptide of the present invention, variant polynucleotides and polypeptides preferably have additional structural and/or functional features in common with a polynucleotide of the present invention. Polynucleotides having a specified degree of identity to, or capable of hybridizing to, a polynucleotide of the present invention preferably additionally have at least one of the following features: (1) they contain an open reading frame, or partial open reading frame, encoding a polypeptide, or a functional portion of a polypeptide, having substantially the same functional properties as the polypeptide, or functional portion thereof, encoded by a polynucleotide in a recited SEQ ID NO:; or (2) they contain identifiable domains in common. Similarly, polypeptides having a specified degree of identity to a polypeptide of the present invention preferably additionally have at least one of the following features: (1) they have substantially the same functional properties as the polypeptide in the recited SEQ ID NO:; or (2) they contain identifiable domains in common.

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Polynucleotide or polypeptide sequences may be aligned, and percentages of identical nucleotides or amino acids in a specified region may be determined against another

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polynucleotide or polypeptide, using computer algorithms that are publicly available. The BLASTN and FASTA algorithms, set to the default parameters described in the documentation and distributed with the algorithm, may be used for aligning and identifying the similarity of polynucleotide sequences. The alignment and similarity of polypeptide sequences may be examined using the BLASTP algorithm. BLASTX and FASTX algorithms compare nucleotide query sequences translated in all reading frames against polypeptide sequences. The FASTA and FASTX algorithms are described in Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444-2448, 1988; and in Pearson, Methods in Enzymol. 183:63-98, 1990. The FASTA software package is available from the University of Virginia by contacting the Assistant Provost for Research, University of Virginia, PO Box 9025, Charlottesville, VA 22906-9025. The BLASTN software is available from the National Center for Biotechnology Information (NCBI), National Library of Medicine, Building 38A, Room 8N805, Bethesda, MD 20894. The BLASTN algorithm Version 2.0.11 [Jan-20-2000] and Version 2.2.1 [Apr-13-2001] set to the default parameters described in the documentation and distributed with the algorithm, are preferred for use in the determination of polynucleotide variants according to the present invention. The use of the BLAST family of algorithms, including BLASTN, BLASTP and BLASTX, is described in the publication of Altschul et al., "Gapped BLAST and PSI-BLAST: a new generation of protein database search programs," Nucleic Acids Res. 25:3389-3402, 1997.

The following running parameters are preferred for determination of alignments and similarities using BLASTN that contribute to the E values and percentage identity for polynucleotides: Unix running command with the following default parameters: blastall -p blastn -d embldb -e 10 -G 0 -E 0 -r 1 -v 30 -b 30 -i queryseq -o results; and parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -r Reward for a nucleotide match (BLASTN only) [Integer]; -v Number of one-line descriptions (V) [Integer]; -b Number of alignments to show (B) [Integer]; -i Query File [File In]; -o BLAST report Output File [File Out] Optional.

The following running parameters are preferred for determination of alignments and similarities using BLASTP that contribute to the E values and percentage identity of

polypeptide sequences: blastall –p blastp –d swissprotdb –e 10 -G 0 -E 0 –v 30 –b 30 –i queryseq –o results; the parameters are: -p Program Name [String]; -d Database [String]; -e Expectation value (E) [Real]; -G Cost to open a gap (zero invokes default behavior) [Integer]; -E Cost to extend a gap (zero invokes default behavior) [Integer]; -v Number of one-line descriptions (v) [Integer]; -b Number of alignments to show (b) [Integer]; -I Query File [File In]; -o BLAST report Output File [File Out] Optional.

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The "hits" to one or more database sequences by a queried sequence produced by BLASTN, BLASTP, FASTA, or a similar algorithm, align and identify similar portions of sequences. The hits are arranged in order of the degree of similarity and the length of sequence overlap. Hits to a database sequence generally represent an overlap over only a fraction of the sequence length of the queried sequence.

As noted above, the percentage identity of a polynucleotide or polypeptide sequence is determined by aligning polynucleotide and polypeptide sequences using appropriate algorithms, such as BLASTN or BLASTP, respectively, set to default parameters; identifying the number of identical nucleic or amino acids over the aligned portions; dividing the number of identical nucleic or amino acids by the total number of nucleic or amino acids of the polynucleotide or polypeptide of the present invention; and then multiplying by 100 to determine the percentage identity. By way of example, a queried polynucleotide having 220 nucleic acids has a hit to a polynucleotide sequence in the EMBL database having 520 nucleic acids over a stretch of 23 nucleotides in the alignment produced by the BLASTN algorithm using the default parameters. The 23-nucleotide hit includes 21 identical nucleotides, one gap and one different nucleotide. The percentage identity of the queried polynucleotide to the hit in the EMBL database is thus 21/220 times 100, or 9.5%. The percentage identity of polypeptide sequences may be determined in a similar fashion.

The BLASTN and BLASTX algorithms also produce "Expect" values for polynucleotide and polypeptide alignments. The Expect value (E) indicates the number of hits one can "expect" to see over a certain number of contiguous sequences by chance when searching a database of a certain size. The Expect value is used as a significance threshold for determining whether the hit to a database indicates true similarity. For example, an E value of 0.1 assigned to a polynucleotide hit is interpreted as meaning that in a database of the size of the EMBL database, one might expect to see 0.1 matches over the aligned portion

of the sequence with a similar score simply by chance. By this criterion, the aligned and matched portions of the sequences then have a probability of 90% of being related. For sequences having an E value of 0.01 or less over aligned and matched portions, the probability of finding a match by chance in the EMBL database is 1% or less using the BLASTN algorithm. E values for polypeptide sequences may be determined in a similar fashion using various polypeptide databases, such as the SwissProt database.

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According to one embodiment, "variant" polynucleotides and polypeptides, with reference to each of the polynucleotides and polypeptides of the present invention, preferably comprise sequences having the same number or fewer nucleotides or amino acids than each of the polynucleotides or polypeptides of the present invention and producing an E value of 0.01 or less when compared to the polynucleotide or polypeptide of the present invention. That is, a variant polynucleotide or polypeptide is any sequence that has at least a 99%. probability of being related to the polynucleotide or polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTN or BLASTX algorithms set at the default parameters. According to a preferred embodiment, a variant polynucleotide is a sequence having the same number or fewer nucleic acids than a polynucleotide of the present invention that has at least a 99% probability of being related to the polynucleotide of the present invention, measured as having an E value of 0.01 or less using the BLASTN algorithm set at the default parameters. Similarly, according to a preferred embodiment, a variant polypeptide is a sequence having the same number or fewer amino acids than a polypeptide of the present invention that has at least a 99% probability of being related as the polypeptide of the present invention, measured as having an E value of 0.01 or less using the BLASTP algorithm set at the default parameters.

In an alternative embodiment, variant polynucleotides are sequences that hybridize to a polynucleotide of the present invention under stringent conditions. Stringent hybridization conditions for determining complementarity include salt conditions of less than about 1 M, more usually less than about 500 mM, and preferably less than about 200 mM. Hybridization temperatures can be as low as 5°C, but are generally greater than about 22°C, more preferably greater than about 30°C, and most preferably greater than about 37°C. Longer DNA fragments may require higher hybridization temperatures for specific hybridization. Since the stringency of hybridization may be affected by other factors such as

probe composition, presence of organic solvents, and extent of base mismatching, the combination of parameters is more important than the absolute measure of any one alone. An example of "stringent conditions" is prewashing in a solution of 6X SSC, 0.2% SDS; hybridizing at 65°C, 6X SSC, 0.2% SDS overnight; followed by two washes of 30 minutes each in 1X SSC, 0.1% SDS at 65°C and two washes of 30 minutes each in 0.2X SSC, 0.1% SDS at 65°C.

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The present invention also encompasses polynucleotides that differ from the disclosed sequences but that, as a consequence of the discrepancy of the genetic code, encode a polypeptide having similar enzymatic activity to a polypeptide encoded by a polynucleotide of the present invention. Thus, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NO: 1-62 and 125-162, or complements, reverse sequences, or reverse complements of those sequences, as a result of conservative substitutions are contemplated by and encompassed within the present invention. Additionally, polynucleotides comprising sequences that differ from the polynucleotide sequences recited in SEQ ID NO: 1-62 and 125-162, or complements, reverse complements or reverse sequences thereof, as a result of deletions and/or insertions totaling less than 10% of the total sequence length are also contemplated by and encompassed within the present invention. Similarly, polypeptides comprising sequences that differ from the polypeptide sequences recited in SEQ ID NO: 63-124 and 163-190 as a result of amino acid substitutions, insertions, and/or deletions totaling less than 10% of the total sequence length are contemplated by and encompassed within the present invention, provided the variant polypeptide has activity in a lignin, fructan or tannin biosynthetic pathway.

In another aspect, the present invention provides genetic constructs comprising, in the 5'-3' direction, a gene promoter sequence; an open reading frame coding for at least a functional portion of a polypeptide of the present invention; and a gene termination sequence. The open reading frame may be orientated in either a sense or anti-sense direction. For applications where amplification of lignin, fructan or tannin synthesis is desired, the open reading frame may be inserted in the construct in a sense orientation, such that transformation of a target organism with the construct will lead to an increase in the number of copies of the gene and therefore an increase in the amount of enzyme. When down-regulation of lignin, fructan or tannin synthesis is desired, the open reading frame may be

inserted in the construct in an anti-sense orientation, such that the RNA produced by transcription of the polynucleotide is complementary to the endogenous mRNA sequence. This, in turn, will result in a decrease in the number of copies of the gene and therefore a decrease in the amount of enzyme. Alternatively, regulation may be achieved by inserting appropriate sequences or subsequences (e.g., DNA or RNA) in ribozyme constructs.

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Genetic constructs comprising a non-coding region of a gene coding for a polypeptide of the present invention, or a nucleotide sequence complementary to a non-coding region, together with a gene promoter sequence and a gene termination sequence, are also provided. As used herein the term "non-coding region" includes both transcribed sequences which are not translated, and non-transcribed sequences within about 2000 base pairs 5' or 3' of the translated sequences or open reading frames. Examples of non-coding regions which may be usefully employed in the inventive constructs include introns and 5'- non-coding leader sequences. Transformation of a target plant with such a genetic construct may lead to a reduction in the amount of lignin, fructan or tannin synthesized by the plant by the process of cosuppression, in a manner similar to that discussed, for example, by Napoli *et al.*, *Plant Cell* 2:279-290, 1990; and de Carvalho Niebel *et al.*, *Plant Cell* 7:347-358, 1995.

The genetic constructs of the present invention further comprise a gene promoter sequence and a gene termination sequence, operably linked to the polynucleotide to be transcribed, which control expression of the gene. The gene promoter sequence is generally positioned at the 5' end of the polynucleotide to be transcribed, and is employed to initiate transcription of the polynucleotide. Gene promoter sequences are generally found in the 5' non-coding region of a gene but they may exist in introns (Luehrsen, *Mol. Gen. Genet.* 225:81-93, 1991). When the construct includes an open reading frame in a sense orientation, the gene promoter sequence also initiates translation of the open reading frame. For genetic constructs comprising either an open reading frame in an anti-sense orientation or a non-coding region, the gene promoter sequence consists only of a transcription initiation site having a RNA polymerase binding site.

A variety of gene promoter sequences which may be usefully employed in the genetic constructs of the present invention are well known in the art. The promoter gene sequence, and also the gene termination sequence, may be endogenous to the target plant host or may be exogenous, provided the promoter is functional in the target host. For example, the

promoter and termination sequences may be from other plant species, plant viruses, bacterial plasmids and the like. Preferably, gene promoter and termination sequences are from the inventive sequences themselves.

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Factors influencing the choice of promoter include the desired tissue specificity of the construct, and the timing of transcription and translation. For example, constitutive promoters, such as the 35S Cauliflower Mosaic Virus (CaMV 35S) promoter, will affect the activity of the enzyme in all parts of the plant. Use of a tissue specific promoter will result in production of the desired sense or anti-sense RNA only in the tissue of interest. With DNA constructs employing inducible gene promoter sequences, the rate of RNA polymerase binding and initiation can be modulated by external stimuli, such as light, heat, anaerobic stress, alteration in nutrient conditions and the like. Temporally regulated promoters can be employed to effect modulation of the rate of RNA polymerase binding and initiation at a specific time during development of a transformed cell. Preferably, the original promoters from the enzyme gene in question, or promoters from a specific tissue-targeted gene in the organism to be transformed, such as Lolium or Festuca, are used. Grass promoters different from the original gene may also be usefully employed in the inventive genetic constructs in order to prevent feedback inhibition. For example, the fructosyltransferase gene will be regulated by sucrose sensing systems; therefore removing the gene from under control of its normal promoter allows the gene to be active all the time. Other examples of gene promoters which may be usefully employed in the present invention include, mannopine synthase (mas), octopine synthase (ocs) and those reviewed by Chua et al., Science 244:174-181, 1989.

The gene termination sequence, which is located 3' to the polynucleotide to be transcribed, may come from the same gene as the gene promoter sequence or may be from a different gene. Many gene termination sequences known in the art may be usefully employed in the present invention, such as the 3' end of the *Agrobacterium tumefaciens* nopaline synthase gene. However, preferred gene terminator sequences are those from the original enzyme gene or from the target species to be transformed.

The genetic constructs of the present invention may also contain a selection marker that is effective in plant cells, to allow for the detection of transformed cells containing the inventive construct. Such markers, which are well known in the art, typically confer

resistance to one or more toxins. One example of such a marker is the NPTII gene whose expression results in resistance to kanamycin or hygromycin, antibiotics which are usually toxic to plant cells at a moderate concentration (Rogers et al., in Weissbach A and H, eds., Methods for Plant Molecular Biology, Academic Press Inc.: San Diego, CA, 1988). Alternatively, the presence of the desired construct in transformed cells can be determined by means of other techniques well known in the art, such as Southern and Western blots.

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Techniques for operatively linking the components of the inventive genetic constructs are well known in the art and include the use of synthetic linkers containing one or more restriction endonuclease sites as described, for example, by Sambrook *et al.*, (*Molecular cloning: a laboratory manual*, CSHL Press: Cold Spring Harbor, NY, 1989). The genetic construct of the present invention may be linked to a vector having at least one replication system, for example, *E. coli*, whereby after each manipulation, the resulting construct can be cloned and sequenced and the correctness of the manipulation determined.

The genetic constructs of the present invention may be used to transform a variety of plants, both monocotyledonous (e.g., grasses, maize/corn, grains, oats, rice, sorghum, millet, rye, sugar cane, wheat and barley), dicotyledonous (e.g., Arabidopsis, tobacco, legumes, alfalfa, oaks, eucalyptus, maple), and gymnosperms. In a preferred embodiment, the inventive genetic constructs are employed to transform grasses. Preferably the target plant is selected from the group consisting of Lolium and Festuca species, most preferably from the group consisting of Lolium perenne and Festuca arundinacea. Other plants that may be usefully transformed with the inventive genetic constructs include other species of ryegrass and fescue, including, but not limited to Lolium multiflorum (Italian ryegrass), Lolium hybridum (hybrid ryegrass), Lolium rigidum (Wimerra grass), Lolium temulentum (darnel), Festuca rubra (red fescue) and Festuca pratensis (meadow fescue). As discussed above, transformation of a plant with a genetic construct of the present invention will produce a modified lignin, fructan or tannin content in the plant.

The production of RNA in target cells may be controlled by choice of the promoter sequence, or by selecting the number of functional copies or the site of integration of the polynucleotides incorporated into the genome of the target organism. A target plant may be transformed with more than one construct of the present invention, thereby modulating the lignin, fructan and/or tannin biosynthetic pathways by affecting the activity of more than one

enzyme, affecting enzyme activity in more than one tissue or affecting enzyme activity at more than one expression time. Similarly, a construct may be assembled containing more than one open reading frame coding for an enzyme encoded by a polynucleotide of the present invention or more than one non-coding region of a gene coding for such an enzyme. The polynucleotides of the present invention may also be employed in combination with other known sequences encoding enzymes involved in the lignin, fructan and/or tannin biosynthetic pathways. In this manner, more than one biosynthetic pathway may be modulated, or a lignin, fructan or tannin biosynthetic pathway may be added to a plant to produce a plant having an altered phenotype.

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Techniques for stably incorporating genetic constructs into the genome of target plants are well known in the art and include Agrobacterium tumefaciens mediated introduction, electroporation, protoplast fusion, injection into reproductive organs, injection into immature embryos, high velocity projectile introduction and the like. The choice of technique will depend upon the target plant to be transformed. For example, dicotyledonous plants and certain monocots and gymnosperms may be transformed by Agrobacterium Ti plasmid technology, as described, for example by Bevan, Nucleic Acid Res. 12:8711-8721, 1984. Targets for the introduction of the genetic constructs of the present invention include tissues, such as leaf tissue, disseminated cells, protoplasts, seeds, embryos, meristematic regions; cotyledons, hypocotyls, and the like. Transformation techniques which may be usefully employed in the inventive methods include those taught by Ellis et al., Plant Cell Reports, 8:16-20, 1989; Wilson et al., Plant Cell Reports 7:704-707, 1989; Tautorus et al., Theor. Appl. Genet. 78:531-536, 198; Hiei et al., Plant J. 6:271-282, 1994; and Ishida et al., Nature Biotechnol. 14:745-750, 1996; US Patent 5,591,616; and European Patent Publication EP 672 752 A1. Once the cells are transformed, cells having the inventive DNA construct incorporated in their genome may be selected by means of a marker, such as the kanamycin resistance marker discussed above. Transgenic cells may then be cultured in an appropriate medium to regenerate whole plants, using techniques well known in the art. In the case of protoplasts, the cell wall is allowed to reform under appropriate osmotic conditions. In the case of seeds or embryos, an appropriate germination or callus initiation medium is employed. For explants, an appropriate regeneration medium is used. Regeneration of plants is well established for many species. The resulting transformed plants may be reproduced

sexually or asexually, using methods well known in the art, to give successive generations of transgenic plants.

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Polynucleotides of the present invention may also be used to specifically suppress gene expression by methods that operate post-transcriptionally to block the synthesis of products of targeted genes, such as RNA interference (RNAi), and quelling. For a review of techniques of gene suppression see *Science*, 288:1370-1372, 2000. Exemplary gene silencing methods are also provided in WO 99/49029 and WO 99/53050. Posttranscriptional gene silencing is brought about by a sequence-specific RNA degradation process which results in the rapid degradation of transcripts of sequence-related genes. Studies have provided evidence that double-stranded RNA may act as a mediator of sequence-specific gene silencing (see, e.g., review by Montgomery and Fire, *Trends in Genetics*, 14: 255-258, 1998). Gene constructs that produce transcripts with self-complementary regions are particularly efficient at gene silencing. A unique feature of this posttranscriptional gene silencing pathway is that silencing is not limited to the cells where it is initiated. The gene-silencing effects may be disseminated to other parts of an organism and even transmitted through the germ line to several generations.

The polynucleotides of the present invention may be employed to generate gene silencing constructs and or gene-specific self-complementary RNA sequences that can be delivered by conventional art-known methods to plant tissues, such as forage grass tissues. Within genetic constructs, sense and antisense sequences can be placed in regions flanking an intron sequence in proper splicing orientation with donor and acceptor splicing sites, such that intron sequences are removed during processing of the transcript and sense and antisense sequences, as well as splice junction sequences, bind together to form double-stranded RNA. Alternatively, spacer sequences of various lengths may be employed to separate self-complementary regions of sequence in the construct. During processing of the gene construct transcript, intron sequences are spliced-out, allowing sense and anti-sense sequences, as well as splice junction sequences, to bind forming double-stranded RNA. Select ribonucleases bind to and cleave the double-stranded RNA, thereby initiating the cascade of events leading to degradation of specific mRNA gene sequences, and silencing specific genes. Alternatively, rather than using a gene construct to express the self-complementary RNA sequences, the gene-specific double-stranded RNA segments are

delivered to one or more targeted areas to be internalized into the cell cytoplasm to exert a gene silencing effect. Gene silencing RNA sequences comprising the polynucleotides of the present invention are useful for creating genetically modified plants with desired phenotypes as well as for characterizing genes (e.g., in high-throughput screening of sequences), and studying their functions in intact organisms.

Example 1 ISOLATION OF CDNA SEQUENCES FROM L. PERENNE AND F. ARUNDINACEA CDNA LIBRARIES

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L. perenne and F. arundinacea cDNA expression libraries were constructed and screened as follows. Tissue was collected from L. perenne and F. arundinacea during winter and spring, and snap-frozen in liquid nitrogen. The tissues collected include those obtained from leaf blades, leaf base, pseudostem, floral stems, inflorescences, roots and stem. Total RNA was isolated from each tissue type using TRIzol Reagent (BRL Life Technologies, Gaithersburg, MD). mRNA from each tissue type was obtained using a Poly(A) Quik mRNA isolation kit (Stratagene, La Jolla, CA), according to the manufacturer's specifications. cDNA expression libraries were constructed from the purified mRNA by reverse transcriptase synthesis followed by insertion of the resulting cDNA in Lambda ZAP using a ZAP Express cDNA Synthesis Kit (Stratagene, La Jolla, CA), according to the manufacturer's protocol. The resulting cDNA clones were packaged using a Gigapack Π Packaging Extract (Stratagene, La Jolla, CA) employing 1 μ l of sample DNA from the 5 μ l ligation mix. Mass excision of the libraries was done using XL1-Blue MRF' cells and XLOLR cells (Stratagene, La Jolla, CA) with ExAssist helper phage (Stratagene, La Jolla, CA). The excized phagemids were diluted with NZY broth (Gibco BRL, Gaithersburg, MD) and plated out onto LB-kanamycin agar plates containing 5-bromo-4-chloro-3-indolyl-beta-D-galactosidase (X-gal) and isopropylthio-beta-galactoside (IPTG). Of the colonies plated and picked for DNA preparations, the large majority contained an insert suitable for sequencing. Positive colonies were cultured in NZY broth with kanamycin and DNA was purified following standard protocols. Agarose gel at 1% was used to screen sequencing templates for chromosomal contamination. Dye terminator sequences were prepared using a

Biomek 2000 robot (Beckman Coulter Inc., Fullerton, CA) for liquid handling and DNA amplification using a 9700 PCR machine (Perkin Elmer/Applied Biosystems, Foster City, CA) according to the manufacturer's protocol.

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The DNA sequences for positive clones were obtained using a Perkin Elmer/Applied Biosystems Division Prism 377 sequencer. cDNA clones were sequenced from the 5' end. The polynucleotide sequences identified as SEQ ID NO: 4, 6, 11, 127, 128 and 132 were identified from L. perenne leaf cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 1, 14, 15, 26, 32, 36, 38, 41, 49, 125, 134, 141, 144, 147, and 150 were identified from L. perenne vegetative stem cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 17, 22, 25, 138, and 140 were identified from L. perenne leaf and pseudostem cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 43, 57, 61, 157, and 161 were identified from L. perenne pseudostem cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 10, 12, 28, 30, 34, 44, 60, 131, 133, 142, 143, 145, 151, and 160 were identified from L. perenne floral stem cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 8, 18, 46, 52, 53, 55, 59, 136, 152, 155, 156, 159, and 162 were identified from L. perenne stem cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 51 and 154 were identified from a L. perenne root cDNA expression library; the polynucleotide sequences identified as SEQ ID NO: 24, 27 and 139 were identified from L. perenne leaf blade cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 9, 37, 39, 40, 45, 130, 148, and 149 were identified from F. arundinacea basal leaf cDNA expression libraries; the polynucleotide sequences identified as SEQ ID NO: 19, 21, 29, 33, 35, 47, 48, and 153 were identified from F. arundinacea combined day 3 and day 6 basal leaves cDNA expression libraries; the polynucleotide sequence identified as SEQ ID NO: 54 was identified from a F. arundinacea combined day 3 and day 6 leaves cDNA expression library; the polynucleotide sequence identified as SEQ ID NO: 56 was identified from a F. arundinacea inflorescence cDNA expression library; the polynucleotide sequences identified as SEQ ID NO: 20 and 137 were identified from a subtracted F. arundinacea leaf blade cDNA expression library; the polynucleotide sequences identified as SEQ ID NO: 7, 23, 42, 50, 62, and 129 were identified from F. arundinacea pseudostem cDNA expression libraries; the polynucleotide

sequences identified as SEQ ID NO: 2, 13, 16 and 135 were identified from *F. arundinacea* leaf cDNA expression libraries; and the polynucleotide sequences identified as SEQ ID NO: 3, 5, 31, and 126 were identified from a *F. arundinacea* inflorescence day 0 cDNA expression library.

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BLASTN Polynucleotide Analysis

The isolated cDNA sequences were compared to sequences in the EMBL DNA database using the computer algorithm BLASTN. Comparisons of DNA sequences provided in SEQ ID NOS: 1-62 to sequences in the EMBL DNA database were made as of October 19, 2001 using BLASTN algorithm Version 2.0.11 [Jan-20-2000], and the following Unix running command: blastall –p blastn –d embldb –e 10 –G0 –E0 –r 1 –v 30 –b 30 –i queryseq –o. Comparisons of DNA sequences provided in SEQ ID NOS: 125-162 to sequences in the EMBL DNA database were made using BLASTN algorithm Version 2.2.1 [Apr-13-2001], and the following Unix running command: blastall –p blastn –d embldb -F F –e 10 –G0 –E0 –r 1 –v 2 –b 2 –i queryseq –o.

The sequences of SEQ ID NO: 4-6, 9-11, 17-19, 21-26, 33, 44, 45, 48, 49, 51-55, 59, 60, 130-132, 136, 139, 146, 151, 154-156, 159, and 162 were determined to have less than 50% identity to sequences in the EMBL database using the computer algorithm BLASTN, as described above. The sequences of SEQ ID NO: 2, 3, 7, 8, 14, 16, 36-38, 46, 47, 50, 56-58, 61, 129, 135, 137, 138, 152, 153, 157, 158, 160 and 161 were determined to have less than 75% identity to sequences in the EMBL database using the computer algorithm BLASTN, as described above. The sequences of SEQ ID NOS: 1, 12, 13, 15, 20, 28, 31, 32, 35, 40 62, 125-128, 133, 134, 142, 144 and 147 were determined to have less than 90% identity to sequences in the EMBL database using the computer algorithm BLASTN, as described above. Finally, the sequences of SEQ ID NOS: 29, 30, 39, 41-43, 141, 143, 148, and 149 were determined to have less than 98% identity to sequences in the EMBL database using the computer algorithm BLASTN, as described above.

BLASTP Polypeptide Analysis

The protein sequences corresponding to the isolated cDNA sequences were compared to sequences in the SwissProt/Trembl protein database using the computer algorithm

BLASTP. Comparisons of protein sequences provided in SEQ ID NOS: 63-124 to sequences in the SwissProt/Trembl protein database were made as of October 19, 2001 using BLASTP algorithm Version 2.0.11 [Jan-20-2000], and the following Unix running command: blastall –p blastp –dstdb–e 10 –G0 –E0 –v 30 –b 30 –i queryseq –o. Comparisons of protein sequences provided in SEQ ID NOS: 163-190 to sequences in the SwissProt/Trembl protein database were made using BLASTP algorithm Version 2.2.1 [Apr-13-2001], and the following Unix running command: blastall –p blastp –d stdb -F F –e 10 –G0 –E0 –v 2 –b 2 – i queryseq –o.

The sequences of SEQ ID NOS: 65-68, 72, 73, 78, 80, 81, 84, 85, 87, 88, 106, 107, 110,111, 113-115, 117, 118 and 121 were determined to have less than 50% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTP, as described above. The sequences of SEQ ID NOS: 71, 79, 82, 83, 86, 95, 98-100, 112, 116, 120, 122-124, 167, 168, 171-174, 185, 188, and 190 were determined to have less than 75% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTP, as described above. The sequences of SEQ ID NOS: 63, 64, 69, 70, 74-77, 90, 91, 93, 94, 97, 101, 102, 104, 108, 109, 119, 175, 183, 187, and 189 were determined to have less than 90% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTP, as described above. Finally, the sequences of SEQ ID NOS: 89, 92, 96, 103, 105, 163-165, 169, 170, 177, 179, 181, 184, and 186 were determined to have less than 98% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTP, as described above.

BLASTX Polynucleotide Analysis

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The isolated cDNA sequences were compared to sequences in the SwissProt/Trembl protein database using the computer algorithm BLASTX. Comparisons of DNA sequences provided in SEQ ID NOS: 1-62 to sequences in the SwissProt/Trembl protein database were made as of October 19, 2001 using BLASTX algorithm Version 2.0.11 [Jan-20-2000], and the following Unix running command: blastall –p blastx –dstdb –e 10 –G0 –E0 –v 30 –b 30 –i queryseq –o. Comparisons of DNA sequences provided in SEQ ID NOS: 1-62 to sequences in the SwissProt/Trembl protein database were made using BLASTX algorithm

Version 2.2.1 [Apr-13-2001], and the following Unix running command: blastall –p blastx – d stdb -F F –e 10 –G0 –E0 –v 2 –b 2 –i queryseq –o.

The sequences of SEQ ID NOS: 11, 44, 45, 48, 49, 51, 52, 55, 130, 132, 155, 156, and 162 were determined to have less than 50% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTX, as described above. The sequences of SEQ ID NOS: 3-10, 16-26, 33, 36-38, 40-43, 50, 53, 54, 56, 58-62, 129, 131, 135-139, 146, 150, 151, 154, and 158-161 were determined to have less than 75% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTX, as described above. The sequences of SEQ ID NOS: 1, 2, 12-15, 27, 28-32, 34, 35, 39, 46, 47, 57, 125-128, 133, 134, 141-145, 147-149, 152, 153, and 157 were determined to have less than 90% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTX, as described above. Finally, the sequence of SEQ ID NO: 140 was determined to have less than 98% identity to sequences in the SwissProt/Trembl database using the computer algorithm BLASTX, as described above.

The location of open reading frames (ORFs), by nucleotide position, contained within the sequences of SEQ ID NO: 1-62 and 125-162, and the corresponding amino acid sequences are provided in Table 2 below. SEQ ID NO: 1-8, 10-15, 17, 19, 21, 23-25, 28-52, 54-59, 61-62 and 125-162 are believed to contain full-length ORFs.

20 TABLE 2

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POLYNUCLEOTIDE SEQ ID NO:	ORF	POLYPEPTIDE SEQ ID NO:	
1	56-2,020	63	
2	64-2,010	64	
3	64-1,926	65	
4	74-1,945	66	
5	40-1,911	67	
6	79-1,938	68	
7	246-1,514	69	
8	264-1,532	70	
9	84-3,272	71	
10	73-3,297	72	
11	129-2,942	73	
12	46-2,472	74	
13	113-2,539	75	
14	61-2,505	76	

POLYNUCLEOTIDE ORF		POLYPEPTIDE SEQ ID NO:
SEQ ID NO:	103-2,253	77
	3-1,439	78
16	26-1,777	79
17		80
18	2-1,174 59-1,852	81
19		82
20	2-1,201 1-1,779	83
21		84
22	198-1,097	85
23	27-1,772	86
24	36-1,802	
25	78-2,084	87
26	2-1,423	88
27	3-1,622	89
28	85-1,764	90
29	72-1,751	91
30	127-1,800	92
31	137-1,810	93
32	62-1,567	94
33	80-1,597	95
34	32-1,117	96
. 35	86-1,171	97
36	55-852	98
37	75-872	99
38	149-1,240	100
39	90-1,118	101
40	28-1,110	102
41	66-1,148	103
42	64-1,146	104
43	85-1,170	105
44	88-1,683	106
45	93-1,721	107
46	111-2,246	108
47	144-2,285	109
48	22-993	110
49	4-1,038	111
50 .	87-1,067	112
51	59-1,135	113
52	18-1,052	114
53	1-882	115
54	80-1,015	116
55	322-1,014	117
56	172-762	118
57	118-1,299	119

POLYNUCLEOTIDE	JCLEOTIDE ORF	
SEQ ID NO:		SEQ ID NO:
58	5-595	120
59	14-1,003	121
60	1-987	122
61	65-1,174	123
62	103-1,245	124
125	55-2,019	163
126	63-1,925	164
127	73-1,944	165
128	71-1,930	166
131	72-3,299	167
132	134-2,950	168
133	45-2,471	169
134	65-2,512	170
135	74-1,819	171
136	170-1,855	172
137	28-1,770	173
138	26-1,733	174
139	35-1,801	175
140	71-2,083	176
141	63-1,607	177
143	126-1,799	178
144	61-1,566	179
145	67-1,152	180
147	148-1,239	181
149	27-1,109	182
151	87-1,718	183
153	143-2,284	184
156	46-1,017	185
157	117-1,313	186
158	81-1,193	187
159	12-1,001	188
160	26-1,018	189
162	50-1,027	190

SEQ ID NO: 125 and 163 are related to SEQ ID NO: 1 and 63, respectively; SEQ ID NO: 126 and 164 are related to SEQ ID NO: 3 and 65, respectively; SEQ ID NO: 127 and 165 are related to SEQ ID NO: 4 and 66, respectively; SEQ ID NO: 128 and 166 are related to SEQ ID NO: 6 and 68, respectively; SEQ ID NO: 129 is an extended sequence of SEQ ID NO: 7; SEQ ID NO: 130 is an extended sequence of SEQ ID NO: 9; SEQ ID NO: 131 and 167 are related to SEQ ID NO: 10 and 72, respectively; SEQ ID NO: 132 and 168 are related

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to SEQ ID NO: 11 and 73, respectively; SEQ ID NO: 133 and 169 are related to SEQ ID NO: 12 and 74, respectively; SEQ ID NO: 134 and 170 are related to SEQ ID NO: 14 and 76, respectively; SEQ ID NO: 135 and 171 are full-length sequences of SEQ ID NO: 16 and 78, respectively; SEQ ID NO: 136 and 172 are full-length sequences of SEQ ID NO: 18 and 80, respectively; SEQ ID NO: 137 and 173 are related to SEQ ID NO: 20 and 82, respectively; SEQ ID NO: 138 and 174 are full-length sequences of SEQ ID NO: 22 and 84, respectively; SEQ ID NO: 139 and 175 are related to SEQ ID NO: 24 and 86, respectively; SEQ ID NO: 140 and 176 are related to SEQ ID NO: 25 and 87, respectively; SEQ ID NO: 141 and 177 are full-length sequences of SEQ ID NO: 26 and 88, respectively; SEQ ID NO: 142 is related to SEQ ID NO: 28 and encodes the same amino acid sequence; SEQ ID NO: 143 and 178 are related to SEQ ID NO: 30 and 92, respectively; SEQ ID NO: 144 and 179 are related to SEQ ID NO: 32 and 94, respectively; SEQ ID NO: 145 and 180 are full-length sequences of SEQ ID NO: 34 and 96, respectively; SEQ ID NO: 146 is related to SEQ ID NO: 36 and encodes the same amino acid sequence; SEQ ID NO: 147 and 181 are related to SEQ ID NO: 38 and 100, respectively; SEQ ID NO: 148 is related to SEQ ID NO: 39, and encodes the same amino acid sequence; SEQ ID NO: 149 and 182 are related to SEQ ID NO: 40 and 102, respectively; SEQ ID NO: 150 is related to SEQ ID NO: 41 and encodes the same amino acid sequence; SEQ ID NO: 151 and 183 is related to SEQ ID NO: 44 and 106, respectively; SEQ ID NO: 152 is related to SEQ ID NO: 46, and encodes the same amino acid sequence; SEQ ID NO: 153 and 184 are related to SEQ ID NO: 47 and 109, respectively; SEQ ID NO: 154 is related to SEQ ID NO: 51, and encodes the same amino acid sequence; SEQ ID NO: 155 is related to SEQ ID NO: 52, and encodes the same amino acid sequence; SEQ ID NO: 156 and 185 are full-length sequences of SEQ ID NO: 53 and 115, respectively; SEQ ID NO: 162 and 190 are variants of SEQ ID NO: 156 and 185, respectively, with a difference in the 5' region of SEQ ID NO: 156 and 162; SEQ NO: 157 and 186 are related to SEQ ID NO: 57 and 119, respectively; SEQ ID NO: 158 and 187 are related to SEQ ID NO: 58 and 120, respectively; SEQ ID NO: 159 and 188 are full-length sequences of SEQ ID NO: 59 and 121, respectively; SEQ ID NO: 160 and 189 are full-length sequences of SEQ ID NO: 60 and 122, respectively; and SEQ ID NO: 161 is related to SEQ ID NO: 61 and encodes the same amino acid sequence.

Example 2

USE OF SUCROSE PHOSPHATE PHOSPHATASE TO DEPHOSPHORYLATE SUCROSE-6-PHOSPHATE

The *F. arundinacea* and *L. perenne* FaSPP and LpSPP genes (SEQ ID NO: 7 and 8, respectively) share amino acid sequence identity with sucrose-6-phosphate phosphatase genes from other plant species (Lunn *et al.*, *Proc. Natl. Acad. Sci. USA* 97:12914-12919, 2000). These genes were amplified by PCR using the primers given in SEQ ID NO: 191 and 192 to add an initiating methionine, and then cloned into the pET41a expression plasmid. These primers amplified nucleotides 263-1531 and 280-1548 for FaSPP and LpSPP, respectively. The resulting plasmids were transformed into *E. coli* BL21 cells using standard protocols, and protein expression was induced using IPTG.

The soluble recombinant protein was assayed for its ability to specifically dephosphorylate sucrose-6-phosphate (Suc-6-P) but not fructose-6-phosphate (Fru-6-P) using the procedure described by Lunn *et al.* (*ibid.*). The release of phosphate from Suc-6P and Fru-6-P was measured using the Fiske-Subbarow method of determining inorganic phosphate (SIGMA assay kit; Sigma, St Louis, MI), with the change in absorbance at 660 nm being proportional to the amount of phosphate released per unit time. As shown in Fig. 1, both the *Festuca* and *Lolium* SPP enzymes dephosphorylated Suc-6-P but not Fru-6-P, whereas control pET41 extract had no activity on either substrate.

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Example 3

PEROXIDASE ACTIVITY OF GRASS ENZYMES DEMONSTRATED BY THEIR ABILITY TO OXIDIZE 2,2'AZINO-BIS.3-ETHYLBENZYLTHIAZOLINE-6-SULFONIC ACID (ABTS)

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A number of *L. perenne* or *F. arundinacea* genes (SEQ ID NO: 48 – 54) share amino acid identity with peroxidase genes from other plant species (Hiraga *et al.*, *Plant Cell Physiol.* 42:462-468, 2001). The putative amino acid secretion signal sequence was identified by signal Panalysis of the *Lolium* and *Festuca* sequences and homology to known peroxidase proteins. Primers were designed to amplify DNA representing the mature protein (minus signal sequence; Table 3.). These genes were amplified by PCR to add an initiating methionine and then cloned into the pET25b expression plasmid. The resulting plasmid was

transformed into E. coli AD494 (DE3) pLysS cells using standard protocols, and protein expression was induced using IPTG.

TABLE 3

SEQ ID NO DNA	SEQ ID NO PROT	Gene	Primers SEQ ID NO:	DNA bp amplified	Protein codons
50	112	FaPER3	193 194	156-1077	24-326
52	114	LpPER5	195 196	120-1052	35-344

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The insoluble recombinant protein was solubilized and re-folded following protocols described for several recombinant *Arabidopsis* peroxidases (Teilum *et al.*, *Protein Exp. and Purif.* 15:77-82, 1999). The insoluble inclusion bodies within *E. coli* were isolated from lysed cells by standard protocols and the recombinant protein solubilized in 8M urea. The solubilized peroxidase protein was refolded to gain active enzyme by diluting urea to 2M with 5µM Heme, 0.25mM Glutathione reduced, and 0.45mM Glutathione oxidized, pH 8 (20mM Tris-HCl). The refolded protein was used directly to assay peroxidase activity.

Peroxidase activity was measured by incubating recombinant peroxidase with premixed ABTS/H₂O₂ liquid substrate (Sigma, St Louis, MI) and measuring ABTS oxidation by the increase in absorbance at 405nm. Horseradish peroxidase of known activity (Sigma, St Louis, MI) was used as a positive control and boiled samples as a negative control. The results provided in Fig. 2 show that FaPER3 and LpPER5 (SEQ ID NO: 50 and 52, respectively) had similar activity to that of horseradish peroxidase in these assays.

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Example 4 USE OF GRASS FRUCTOSYLTRANSFERASE GENES TO SYNTHESIZE FRUCTANS

Transformation of N. benthamiana plants with fructosyltransferase genes

Sense constructs containing a polynucleotide including the coding region of fructosyltransferase genes isolated from *L. perenne* Lp1-SST and Lp6SFT1 (SEQ ID NO: 125 and 126, respectively) were inserted into a pART27 derived binary vector and used to

transform A. tumefaciens LBA4404 using published methods (see, An et al., "Binary Vectors," in Gelvin and Schilperoort, eds., Plant Molecular Biology Manual, Kluwer Academic Publishers: Dordrecht, 1988). The presence and integrity of the binary vector in A. tumefaciens was verified by polymerase chain reaction (PCR). The primers px17 (SEQ ID NO: 207) and px18 (SEQ ID NO: 208) were used to confirm the presence of the Lp1-SST construct, whereas the primers px19 (SEQ ID NO: 209) and px 20 (SEQ ID NO: 210) were used to confirm the presence of the Lp6-SFT-1 construct.

The A. tumefaciens containing the sense gene constructs were used to transform N. benthamiana leaf discs (Burow et al., Plant Mol. Biol. Report 8:124-139, 1990). Several independent transformed plant lines were established for the sense construct for each fructosyltransferase gene. DNA was isolated from transformed plants containing the appropriate fructosyltransferase gene construct using the QIAGEN DNAeasy Plant Mini Kit (Qiagen, Valencia, CA). Presence of the fructosyltransferase gene was verified using PCR experiments as shown in Figs. 3 and 4. For the Lp6-SFT1 gene, the forward and reverse primers given in SEQ ID NO: 197 and 198 were used, respectively. These primers amplify nucleotides 1572 - 1980 of the Lp6-SFT1 gene which corresponds to a 406 base pair fragment. For Lp1-SST, the forward and reverse primers given in SEQ ID NO: 199 and 200 were used, respectively. These primers amplify nucleotides 1332 - 1740 of Lp1-SST, corresponding to a 414 base pair fragment.

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Effects of fructosyltransferase genes on fructosyltransferase concentration in transformed plants

Fructans are not normally found in N. benthamiana plants; hence, if introduction of the sense fructosyltransferase constructs was successful, it should be possible to extract fructans from the transformed plants. The concentration of fructosyltransferase in the transformed plants was determined using the Fructan Assay Kit (Megazyme International Ireland Ltd, Wicklow, Ireland). Briefly, 300 mg of leaf material from the independent transformed plant lines containing the fructosyltransferase sense constructs were extracted individually at 80 °C with 1 ml 80% ethanol, followed by two 1 ml extractions with water. The ethanol and water extracts were combined and frozen overnight at -20 °C. Extracts were centrifuged at 20,000 g to pellet chlorophyll. Clarified extracts were treated with 1%

PVP-40 to precipitate phenolic compounds. These extracts were then reduced in volume by rotary evaporation.

Fructan levels were determined in these extracts using the Megazyme Fructan Assay kit. Briefly, sucrose, starch and reducing sugars are removed from the plant carbohydrate extracts by using sucrase, β-amylase, pullulanase and maltase, and then converting the resulting reducing sugars to sugar alcohols. The remaining fructans are hydrolyzed with fructanase and the reducing sugars produced (glucose and fructose) are measured by the 4-hydroxybenzoic acid hydrazide (PAHBAH) reducing sugar method. The final extracts are assayed for absorbance at 410 nm. As shown in Fig. 5, fructans could be detected in both the Lp1-SST and Lp6-SFT-1 transgenic lines. Fructan levels were highest in lines 07, 09 and 12 for Lp1-SST, and lines 05 and 12 for Lp6SFT-1.

Example 5

USE OF SUCROSE PHOSPHATE SYNTHASE ENZYMES TO SYNTHESIZE SUCROSE

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A F. arundinacea gene (FaSPS-N; SEQ ID NO: 9) has been identified that shares amino acid sequence identity with sucrose phosphate synthase (SPS) from other plant species. SEQ ID NO: 7 and 8 are also SPS sequences, with SEQ ID NO: 7 being a Lolium perenne homologue of SEQ ID NO: 9. The FaSPS-N was cloned into the pcDNA3 mammalian expression plasmid and the resulting plasmid transfected into 293T mammalian cells (human embryonic kidney derived cells) using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA).

Cell lysates from transfected cells were deionized on G25 spin columns and used in a sucrose synthesis assay. In this assay, mammalian cell extracts were tested for their ability to synthesize sucrose from fructose-6-phosphate and uridine 5'-diphosphoglucose. Following the synthesis reaction, hexoses were converted to sugar alcohols by boiling in the presence of 30% KOH. The sucrose synthesized was detected by the addition of 1.4 % anthrone reagent in H₂SO₄ and incubating at 40 °C for 20 min. The change in absorbance at 620 nm is relative to sucrose in the reaction (Botha and Black, *Aust. J. Plant Physiol.* 27:81-85, 2000). In these experiments, introducing FaSPS-N alone into mammalian cells produced a sucrose synthesis activity that was not detected in non-transfected cells (Fig. 6).

A known cofactor for SPS is SPP. To test whether SPP is required for SPS activity, the *L. perenne* LpSPP gene (SEQ ID NO: 8) was cloned into the pcDNA3 mammalian expression plasmid. Both the FaSPS-N and LpSPP plasmids were co-transfected into 293T mammalian cells using Lipofectamine 2000 reagent (Invitrogen, Carlsbad, CA). Cell lysates from transfected cells were deionized on G25 spin columns and used in a sucrose synthesis assay as described above. As shown in Fig. 6, adding SPP did not significantly enhance or alter the sucrose synthesis activity of the cell extracts.

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Example 6

USE OF SOLUBLE SUCROSE SYNTHASE ENZYMES TO CLEAVE SUCROSE

A F. arundinacea gene (FaSUS-1; SEQ ID NO: 13) was identified that shared amino acid sequence identity with soluble sucrose synthase enzymes (SUS) from other plant species. The FaSUS-1 gene was cloned into the pcDNA3 mammalian expression plasmid, which was transiently transfected into 293T mammalian cells (human embryonic kidney derived cells) using Lipofectamine 2000 reagent (Invitrogen Carlsbad, CA). Transfected cells were grown for several days before harvesting (by scraping cells in a sucrose synthase buffer). Harvested cells were frozen on dry ice and freeze-thawed twice before pelleting cell debris by centrifugation. The resulting supernatant (cell lysate) was deionized on G25 spin columns and then used in a sucrose cleavage assay as described by Sebkova et al. (Plant Physiol. 108:75-83, 1995). In these assays, the cell lysates were tested for their ability to cleave sucrose in the presence of UDP to produce fructose and uridine 5'-diphosphoglucose. Following a 30 min incubation at 30 °C, the enzyme activity was stopped by boiling the tubes for 1 min. Both NAD and UDP-glucose dehydrogenase were added and the change in OD at 340 nM (production of NADPH) was measured. As shown in Fig. 7, significantly higher levels of sucrose cleavage were observed in cells transfected with FaSUS1 construct than in non-transfected control cells.

Example 7

USE OF ACID INVERTASES TO CLEAVE SUCROSE

A number of acid (vacuolar and cell wall) invertase genes from *L. perenne* and *F. arundinacea* (SEQ ID NOS: 17, 19, 21, 23 and 135-141) were identified that share amino acid sequence identity with acid invertases from other plant species (Unger *et al.*, *Plant Physiol.* 104:1351-1357, 1994; Goetz and Roitsch, *J. Plant Physiol.* 157:581-585, 2000). These sequences were analysed by SignalP and homology to identify signal regions and propeptide sequences, and primers were designed to amplify the DNA sequence encoding the mature protein (Table 4).

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TABLE 4

SEQ ID NO DNA	SEQ ID NO PROT	Gene	Primers SEQ ID NO	DNA bp amplified	Protein codons
17	79	LpCWINV1	201 202	137-1803	38-583
19	81	FaCWINV4	203 204	134-1912	26-597
25	87	LpSINV1	205 206	387-2124	104-668

The PCR fragments were cloned into pPICZαA vectors for expression in methylotrophic yeast *Pichia pastoris* (EasySelect TM Pichia Expression Kit, Invitrogen, Carlsbad, CA). The sequences were cloned in frame with the α-mating factor for secretion of the recombinant invertase protein into liquid media, following similar methods described for the expression of barley 6-SFT and fescue 1-SST in *P. pastoris* (Hochstrasser *et al., FEBS Letters* 440:356-360, 1998; Lüscher *et al., Plant Physiol.*, 124:1217-1227, 2000). The media was concentrated 10 fold by Vivaspin 30 kDa spin column (VivaScience, Hannover, Germany) to concentrate recombinant protein and used directly to assay invertase activity. Recombinant protein was assayed with 100mM sucrose in 500 μl phosphate buffer pH5.0, at 30 °C for 1 hour. Release of glucose by invertase activity was measured using a glucose HK assay kit (Sigma, St Louis, MI). Fig. 8 shows the glucose released by invertase activity in terms of glucose concentration in the assay mix. As shown in Fig. 8, invertase activity was observed for the vacuolar invertase (LpSINV1; SEQ NO: 25) and the two cell wall invertases

(LpCWINV1 and FaCWINV4; SEQ NO: 17 and 19, respectively) but not for an empty vector (pPICZalphaA) control.

Example 8

USE OF TANNIN GENES TO MODIFY TANNIN BIOSYNTHESIS

Certain Arabidopsis mutants of the transparent testa (tt) phenotype do not make the anthocyanin pigment cyanidin and therefore have no seed coat color. The genes responsible for many of these mutants have now been identified as shown in Table 5.

TABLE 5

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Enzyme	Abbreviation	Locus	Chromosome
Dihydroflavanol-4-reductase	DFR	tt3	5
Chalcone synthase	CHS	tt4	5
Chalcone isomerase	CHI	tt5	3
Flavanone 3- β-hydroxylase	F 3βH	tt6	3

Over-expression of the maize genes for CHS, CHI and DFR has been shown to complement the *Arabidopsis tt4*, *tt5* and *tt3* mutants, respectively, thereby restoring cyanidin synthesis and seed coat color (Dong *et al.*, *Plant Physiol.* 127:46-57, 2001). Complementation of these *Arabidopsis* mutants may therefore be employed to demonstrate the function of the inventive polynucleotides encoding enzymes involved in the tannin biosynthetic pathway.

Sense constructs containing a polynucleotide including the coding region of tannin genes isolated from *L. perenne* or *F. arundinacea* LpCHS, LpCHI, LpF3βH, LpDFR1, FaCHI and FaF3βH (SEQ ID NO: 157, 55, 161, 159, 56 and 62, respectively) under the control of the CaMV 35S promoter were inserted into a binary vector and used to transform *Agrobacterium tumefaciens* LBA4404 using published methods (*see*, An G, Ebert PR, Mitra A, Ha SB, "Binary Vectors," *in* Gelvin SB, Schilperoort RA, eds., *Plant Molecular Biology Manual*, Kluwer Academic Publishers: Dordrecht, 1988). The presence and integrity of the binary vector in *A. tumefaciens* was verified by polymerase chain reaction (PCR) using the primer pairs described in Table 6.

TABLE 6

Gene	SEQ ID NO:	Transparent testa line	Forward Primer SEQ ID NO:	Reverse Primer SEQ ID NO:
LpCHS	157	tt4	211	212
LpCHI	55	tt5	213	214
LpF3βH	161	tt6	217	218
LpDFR1	159	tt3	215	216
FaCHI	56	tt5	213	214
FaF3βH	62	tt6	217	218

The A. tumefaciens containing the sense gene constructs are used to transform Arabidopsis by floral dipping (Clough and Bent, Plant J. 16:735-743, 1998). Several independent transformed plant lines were established for the sense construct for each of the tannin genes. Specifically, LpDFR1 constructs were transformed into Arabidopsis tt3 mutants, LpCHS constructs were transformed into Arabidopsis tt4 mutants, LpCHI and FaCHI constructs were transformed into Arabidopsis tt5 mutants, and LpF3βH and FaF3βH constructs were transformed into Arabidopsis tt6 mutants. Several independent transformed plant lines were established for the construct for each of the tannin genes. Transformed plants containing the appropriate tannin gene construct were verified using PCR.

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The presence of cyanidin in the FaCHI transformed plants is demonstrated by a phenotypic change in plant seedling color and by analyzing cyanidin extracts made from transgenic plants grown under stressed conditions (Dong et al., Plant Physiol. 127:46-57, 2001). Briefly, cyanidins are extracted from plant tissue with an acid/alcohol solution (HCl/methanol) and water. Chlorophyll is removed by freezing the extracts followed by centrifugation at 4 °C at 20,000 g for 20 min. Any remaining chlorophyll is removed through a chloroform extraction. The absorbance at 530 nm is measured for each of the cyanidin extracts. Non-transgenic wild type and control Arabidopsis plants are used as controls.

SEQ ID NOS: 1-218 are set out in the attached Sequence Listing. The codes for nucleotide sequences used in the attached Sequence Listing, including the symbol "n," conform to WIPO Standard ST.25 (1998), Appendix 2, Table 1.

All references cited herein, including patent references and non-patent publications, are hereby incorporated by reference in their entireties.

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While in the foregoing specification this invention has been described in relation to certain preferred embodiments, and many details have been set forth for purposes of illustration, it will be apparent to those skilled in the art that the invention is susceptible to additional embodiments and that certain of the details described herein may be varied considerably without departing from the basic principles of the invention.

Claims

We claim:

1. An isolated polynucleotide comprising a sequence selected from the group consisting of:

- (a) SEQ ID NO: 1-62 and 125-162;
- (b) complements of SEQ ID NO: 1-62 and 125-162;
- (c) reverse complements of SEQ ID NO: 1-62 and 125-162; and
- (d) reverse sequences of SEQ ID NO: 1-62 and 125-162;
- 2. An isolated polynucleotide comprising a sequence selected from the group consisting of:
 - (a) sequences having a 99% probability of being functionally or evolutionarily related to a sequence of SEQ ID NO: 1-62 and 125-162;
 - (b) sequences having at least 75% identity to a sequence of SEQ ID NO: 1-62 and 125-162:
 - (c) sequences having at least 90% identity to a sequence of SEQ ID NO: 1-62 and 125-162; and
 - (d) sequences having at least 95% identity to a sequence of SEQ ID NO: 1-62 and 125-162,

wherein the polynucleotide encodes a polypeptide having substantially the same functional properties as a polypeptide encoded by SEQ ID NO: 1-62 and 125-162.

- 3. An isolated oligonucleotide probe or primer comprising at least 10 contiguous residues complementary to 10 contiguous residues of a nucleotide sequence recited in claim 1.
- 4. A kit comprising a plurality of oligonucleotide probes or primers of claim 3.
- 5. A genetic construct comprising an isolated polynucleotide of any one of claims 1 and

2.

6. A transgenic cell comprising a construct according to claim 5.

- 7. A construct comprising, in the 5'-3' direction:
 - (a) a gene promoter sequence;
 - (b) a polynucleotide sequence comprising at least one of the following: (1) a polynucleotide coding for at least a functional portion of a polypeptide encoded by a polynucleotide of any one of claims 1 and 2; and (2) a polynucleotide comprising a non-coding region of a polynucleotide of any one of claims 1 and 2; and
 - (c) a gene termination sequence.
- 8. The construct of claim 7, wherein the polynucleotide is in a sense orientation.
- 9. The construct of claim 7, wherein the polynucleotide is in an anti-sense orientation.
- 10. A transgenic plant cell comprising a construct of claim 7.
- 11. A plant comprising a transgenic plant cell according to claim 10, or fruit or seeds or progeny thereof.
- 12. A method for modulating one or more of the lignin composition, fructan composition and tannin composition of a plant, comprising stably incorporating into the genome of the plant at least one polynucleotide of any one of claims 1 and 2.
- 13. The method of claim 12, wherein the plant is selected from the group consisting of grasses.
- 14. The method of claim 13, wherein the plant is selected from the group consisting of: Lolium perenne and Festuca arundinacea.

15. The method of claim 12 comprising stably incorporating into the genome of the plant a construct of claim 7.

- 16. A method for producing a plant having one or more of altered lignin composition, altered fructan composition and altered tannin composition, comprising:
 - (a) transforming a plant cell with a construct of claim 7 to provide a transgenic cell; and
 - (b) cultivating the transgenic cell under conditions conducive to regeneration and mature plant growth.
- 17. A method for modifying the activity of a polypeptide involved in a lignin, fructan or tannin biosynthetic pathway in a plant comprising stably incorporating into the genome of the plant a construct of claim 7.
- 18. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of: SEQ ID NO: 63-124 and 163-190.
- 19. An isolated polypeptide comprising an amino acid sequence selected from the group consisting of:
 - (a) sequences having at least 75% identity to a sequence of SEQ ID NO: 63-124 and 163-190;
 - (b) sequences having at least 90% identity to a sequence of SEQ ID NO: 63-124 and 163-190; and
 - (c) sequences having at least 95% identity to a sequence of SEQ ID NO: 63-124 and 163-190

wherein the polypeptide has substantially the same functional properties as a polypeptide of SEQ ID NO: 63-124 and 163-190.

- 20. An isolated polynucleotide that encodes a polypeptide of claim 18.
- 21. An isolated polypeptide encoded by a polynucleotide of any one of claims 1 and 2.

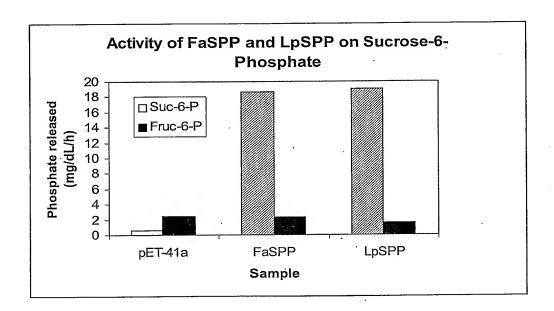


Figure 1.

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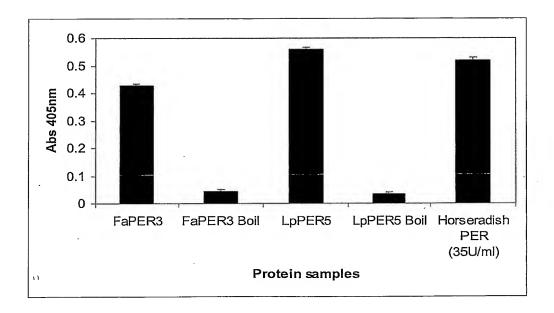


Figure 2.

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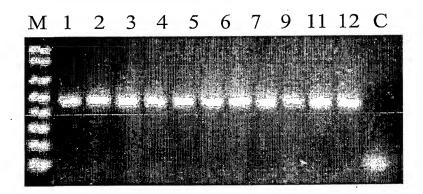


Figure 3.

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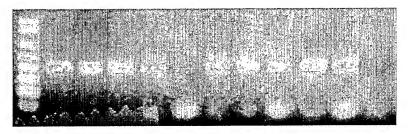
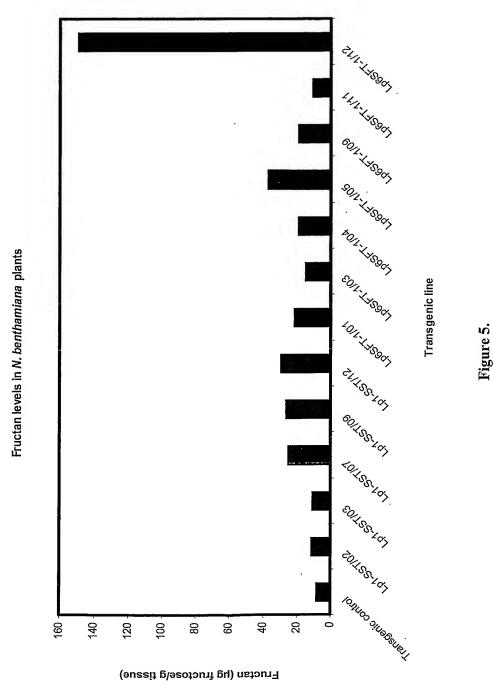


Figure 4.





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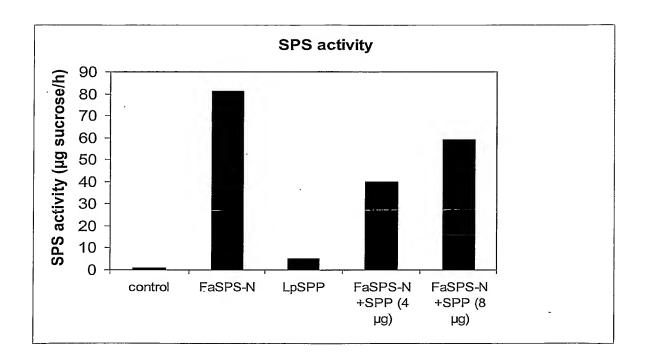


Figure 6.

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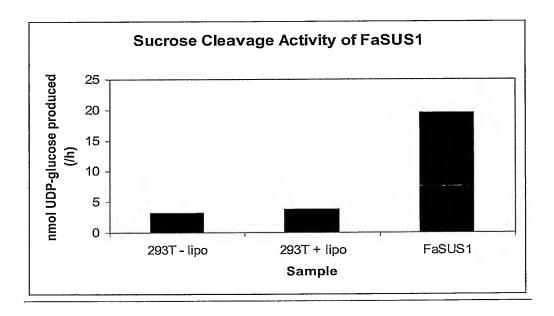


Figure 7.

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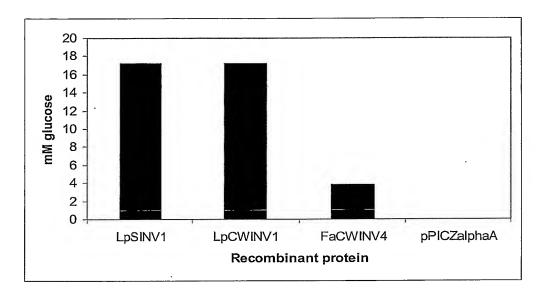


Figure 8.

aaaaaa

2100 2106

SEQUENCE LISTING

<110> Demmer, Jeroen Forster, Richard L Gibson, John Bryan Shenk, Michael Andrew Norriss, Geoffrey Glenn, Matthew Saulsbury, Keith Martin Hall, Claire <120> Compositions isolated from forage grasses and methods for their use <140> <141> 2002-11-07 <160> 218 <170> FastSEQ for Windows Version 4.0 <210> 1 <211> 2106 <212> DNA <213> Lolium perenne 60 120 gtccagcgcc gtcgtccccg gcaccacggc gccgctgctt ccttatgcgt acgcgccgct geogtegtee teegacgacg eeegtgaaaa cagaagtage ggeggegtga ggtggegege 180 gtgcgccgtc gttctggcgg actcggcgtt ggcggtggtg gtcgtggtcg ggctcctcgc 240 300 gggcggcagg gtggatcggg tcccggccgg cgcagacgtg gcgtcggcca cggtgccggc cgtgccgatg gagttcccga ggagccgggg caaggacttg ggcgtgtcgg agaagtcctc 360 cggtgcctac tccgccgacg gcgggttccc gtggagcaac gccatgctgc agtggcagcg 420 480 caccgggttc catttccagc cggagcagca ctacatgaac gatcccaacg gccccgtgta ctacggcgga tggtaccacc tcttctacca gcacaacccc aagggcgaca gctggggcaa 540 categoetgg geccaegecg tgtccaagga catggtcaac tggegecaec tecegetege 600 catggttccc gaccagtggt acgacagcaa cggcgtcctc accggctcca tcaccgtgct 660 ccccgacggc caggtcatcc tgctctacac cggcaacacc gacaccctag cccaggtcca 720 gtgcctcgcc acgcccgccg acccgtccga cccgctcctc cgcgaatgga tcaagcaccc 780 cgccaaccc atcetettee cgccgcccgg gatcgggete aaggaettee gcgacccget 840 900 caccgcctgg ttcgaccact ccgaccacac ctggcgcacc gtcatcgggt ccaaggacga cgacggccac gccggcatca tcctcagcta caagaccaag gacttcgtca actacgagct 960 catgcccggg aacatgcacc gcgggcccga cggcaccggc atgtacgagt gcatcgacct 1020 ctaccccgtc ggcggcaact cgtcggagat gctcggcggc gacgactcgc ccgacgtgct 1080 cttcgtgctc aaggagagca gcgatgacga acgtcacgac tactatgcgc tcggaaggtt 1140 1200 cgacgccgtc gccaacgttt ggacgcccat cgaccgggac ctggaccttg ggatcgggct cagatacgac tggggaaagt actacgcctc caagtccttc tacgaccaga agaagaaccg 1260 ccgcatcgta tgggcataca tcggcgagac cgactccgag caggccgaca tcaccaaggg 1320 atgggccaat ctcatgacga ttccaagaac ggtggagctt gacaggaaga cccgcacaaa cctcatccaa tggccagtgg aggaggtcga caccctccgc aggaactcca cggacctcgg 1380 1440 tegeateace gteaacgeeg geteegteat tegeeteece etecaceagg gegeteaact 1500 cgacatcgag gcctccttcc aactcaactc ttccgacgtg gatgctatca acgaggccga 1560 cgtcggctac aactgcagca ccagcggtgc cgccgtacgg ggggcgctcg gcccctttgg 1620 cctcctcgtc cttgccaatg gccgcaccga acagacggct gtgtacttct acgtgtccaa 1680 gggcgtcgac ggcggcctcc agacccactt ttgccacgac gagtcacggt caacacgggc 1740 aaaggatgtc gtgaatagga tgattggcag catcgtgccg gtgcttgacg gtgagacctt 1800 ttcggtgagg gtgctagtgg accactccat cgtgcagagc ttcgcgatgg gcgggaggat 1860 cacggcgacg tcgcgggcgt acccgacgga gaccatctac gcggccgcag gggtctacct 1920 cttcaacaac gccacgggcg ccaccgtcac cgccgagagg ctcgtcgtgc acgagatggc 1980 ctcagctgac aaccatatct tcacgaacga cgacttgtag atgaaaccaa gtttagctcg 2040

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Glu Glu Val Asp Thr Leu Arg Arg Asn Ser Thr Asp Leu Gly Arg Ile 455 Thr Val Asn Ala Gly Ser Val Ile Arg Leu Pro Leu His Gln Gly Ala 470 475 Gln Leu Asp Ile Glu Ala Ser Phe Gln Leu Asn Ser Ser Asp Val Asp 490 495 485 Ala Ile Asn Glu Ala Asp Val Gly Tyr Asn Cys Ser Thr Ser Gly Ala 500 505 510 Ala Val Arg Gly Ala Leu Gly Pro Phe Gly Leu Leu Val Leu Ala Asn 515 520 525 515 520 Gly Arg Thr Glu Gln Thr Ala Val Tyr Phe Tyr Val Ser Lys Gly Val 530 535 540 Asp Gly Gly Leu Gln Thr His Phe Cys His Asp Glu Ser Arg Ser Thr 555 550 Arg Ala Lys Asp Val Val Asn Arg Met Ile Gly Ser Ile Val Pro Val 575 570 565 Leu Asp Gly Glu Thr Phe Ser Val Arg Val Leu Val Asp His Ser Ile 585 580 Val Gln Ser Phe Ala Met Gly Gly Arg Ile Thr Ala Thr Ser Arg Ala 605 595 600 Tyr Pro Thr Glu Thr Ile Tyr Ala Ala Ala Gly Val Tyr Leu Phe Asn 610 615 620 Asn Ala Thr Gly Ala Thr Val Thr Ala Glu Arg Leu Val Val His Glu 630 635 Met Ala Ser Ala Asp Asn His Ile Phe Thr Asn Asp Asp Leu 650

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<211> 648

<212> PRT <213> Festuca arundinacea

<400> 64

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265 260 Ser Lys Asp Asp Asp Gly His Ala Gly Ile Ile Leu Ser Tyr Lys Thr 285 275 280 Lys Asp Phe Val Asn Tyr Glu Leu Met Pro Gly Asn Met His Arg Gly 295 300 Pro Asp Gly Thr Gly Met Tyr Glu Cys Ile Asp Leu Tyr Pro Val Gly 315 305 310 Gly Asn Ser Ser Glu Met Leu Gly Gly Asp Asp Ser Pro Asp Val Leu 325 330 335 Phe Val Leu Lys Glu Ser Ser Asp Asp Glu Arg His Asp Tyr Tyr Ala 340 345 350 Leu Gly Arg Phe Asp Ala Ala Ala Asn Ile Trp Thr Pro Ile Asp Gln 360 Glu Leu Asp Leu Gly Ile Gly Leu Arg Tyr Asp Trp Gly Lys Tyr Tyr 375 380 Ala Ser Lys Ser Phe Tyr Asp Gln Arg Lys Asn Arg Arg Val Val Trp 390 395 Ala Tyr Ile Gly Glu Thr Asp Ser Glu Gln Ala Asp Ile Thr Lys Gly 405 410 415 Trp Ala Asn Leu Met Thr Ile Pro Arg Thr Val Glu Leu Asp Lys Lys 430 420 425 Thr Arg Thr Asn Leu Ile Gln Trp Pro Val Glu Glu Val Asp Thr Leu 435 440 Arg Arg Asn Ser Thr Asp Leu Gly Arg Ile Thr Val Asn Ala Gly Ser 450 455 460 Val Ile Arg Leu Pro Leu His Gln Gly Ala Gln Leu Asp Ile Glu Ala 475 480 470 Ser Phe Gln Leu Asn Ser Ser Asp Val Asp Ala Leu Asn Glu Ala Asp 485 490 495 Val Gly Tyr Asn Cys Ser Thr Ser Gly Ala Ala Val Arg Gly Ala Leu 500 505 510 Gly Pro Phe Gly Leu Leu Val Leu Ala Asn Gly Arg Thr Glu Gln Thr 515 520 525 Ala Val Tyr Phe Tyr Val Ser Lys Gly Val Asp Gly Ala Leu Gln Thr 540 530 535 His Phe Cys His Asp Glu Ser Arg Ser Thr Arg Ala Lys Asp Val Val 555 560 550 Asn Arg Met Ile Gly Ser Ile Val Pro Val Leu Asp Gly Glu Thr Phe 565 570 575 Ser Val Arg Val Leu Leu Asp His Ser Ile Val Gln Ser Phe Ala Met 580 585 Gly Gly Arg Ile Thr Ala Thr Ser Arg Ala Tyr Pro Thr Glu Ala Ile 595 600 605 Tyr Ala Ala Ala Gly Val Tyr Val Phe Asn Asn Ala Thr Gly Ala Thr 610 615 620 Val Thr Ala Glu Arg Leu Val Val Tyr Glu Met Ala Ser Ala Asp Asn 635 625 630 His Ile Phe Arg Asn Asp Asp Leu

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<212> PRT

<213> Festuca arundinacea

645

<400> 65

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Gly Trp Arg Gly Phe Leu Thr Val Leu Ala Ala Ser Gly Val Val Val 35

Leu Leu Val Ala Ala Thr Met Leu Ala Gly Ser Arg Met Gly Gln Ala 50 55 60

Gly Asp Thr Asp Glu Asp Gly Ala Gly Gly Phe Pro Trp Ser Asn Glu 70 70 75

PCT/NZ02/00239

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Phe	Met	Ser	Asp 100	Pro	Asp	Gly	Pro	Val 105	Tyr	Tyr	Arg	Gly	Tyr 110	Tyr	His
Leu	Phe	Phe 115	Gln	Tyr	Asn	Arg	Arg 120	Gly	Val	Ala	Trp	Asp 125	Asp	Tyr	Ile
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			180					185					11e		
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	290					295					300		Tyr		
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Gly				325	Ala				330				Asp	335	
	_		340					345				-	Leu 350		
		355					360					365	Met		
_	370					375					380		Lys		
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_				405					410				Ala Gly	415	
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465					470					475					480
				485					490					495	Glu
			500					505					510		Leu
		515					520					525			Asp
	530	_				535					540				Glu
545					550					555					Phe 560
				565					570					575	Glu Gly
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<211> 623 ·

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Ile Glu Ala Ser Phe Arg Leu Asp Ala Ser Asp Val Ala Ala Ile Asn 455 460 Glu Ala Asp Val Gly Tyr Asn Cys Ser Ser Ser Gly Gly Ala Ala Ala 470 475 Arg Gly Ala Leu Gly Pro Phe Gly Leu Leu Val His Ala Ala Gly Asp 490 495 485 Leu Arg Gly Glu Gln Thr Ala Val Tyr Phe Tyr Val Ser Arg Ala Leu 500 505 510 Asp Gly Thr Leu Arg Thr Ser Phe Cys Asn Asp Glu Thr Arg Ser Ser 515 520 525 Arg Ala Arg Asp Val Thr Lys Arg Val Val Gly Ser Thr Val Pro Val 530 535 540 Leu Asp Gly Glu Ala Leu Ser Met Arg Val Leu Val Asp His Ser Ile 550 555 Val Gln Ser Phe Ala Met Gly Gly Arg Thr Thr Ala Thr Ser Arg Val 575 565 570 Tyr Pro Thr Glu Ala Ile Tyr Ala Arg Ala Gly Val Tyr Leu Phe Asn 580 585 Asn Ala Thr Gly Ala Gly Val Thr Ala Glu Arg Leu Ile Val His Glu 595 600 605 Met Ala Ser Ala Val Tyr Asp Glu Thr Leu Met Val Glu Asp Ser 615 <210> 67 <211> 623 <212> PRT <213> Festuca arundinacea Met Glu Ser Arg Ala Phe Pro Ser Ala Ala Tyr Ala Pro Leu Leu Pro 1 5 10 Pro Thr Ala Asp Asp Ala Thr Leu Gly Lys Gln Asp Arg Pro Gly Val 20 25 Gly Trp Arg Gly Phe Leu Thr Val Leu Ala Ala Ser Gly Val Val Val 40 45 Leu Leu Val Ala Ala Ser Leu Leu Ala Gly Ser Arg Met Gly Gln Ala 55 Gly Asp Gly Glu Gly Asn Thr Asp Glu Asp Gly Ala Gly Gly Phe Pro 70 75 Trp Ser Asn Glu Met Leu Gln Trp Gln Arg Ala Gly Phe His Tyr Gln 85 90 Pro Glu Gly His Phe Met Ser Asp Pro Asp Gly Pro Val Tyr Tyr Arg 105 110 100 Gly Tyr Tyr His Leu Phe Phe Gln Tyr Asn Arg Arg Gly Val Ala Trp 125 120 Asp Asp Tyr Ile Glu Trp Gly His Val Val Ser Gln Asp Leu Val His 140 135 Trp Arg Pro Leu Pro Val Ala Met Arg Pro Asp His Trp Tyr Asp Lys 145 150 155 Lys Gly Val Leu Ser Gly Thr Ile Thr Val Leu His Asn Gly Thr Leu 170 175 165 Val Leu Leu Tyr Thr Gly Val Thr Glu Asp Pro Met Ala Glu Ser Gln 185 190 180 Cys Ile Ala Val Pro Thr Asp Pro Asn Asn Pro Leu Leu Arg His Trp 200 205 Thr Lys His Pro Ala Asn Pro Val Leu Ala His Pro Gln Gly Val Gln 215 220 Gly Met Asp Phe Arg Asp Pro Thr Ser Ala Trp Phe Asp Lys Ser Asp 230 235 240 Ala Thr Trp Arg Ile Leu Ile Gly Ser Lys Asp Asp Asn Gly Ser 245 250 255 His Ala Gly Ile Ala Phe Ile Phe Lys Thr Lys Asp Phe Leu Ser Phe 260 265 270 265 Glu Arg Val Pro Gly Ile Val His Arg Val Glu Gly Thr Gly Met Trp

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Glu Cys Ile Asp Phe Tyr Pro Val Gly Gly His Asn Ser Ser Ser
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Glu Glu Leu Tyr Val Ile Lys Ala Ser Met Asp Asp Glu Arg His Asp
                     315
               310
Tyr Tyr Ser Leu Gly Arg Tyr Asp Ala Ala Ala Asn Thr Trp Thr Pro
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             325
Leu Asp Ala Glu Leu Asp Leu Gly Ile Gly Leu Arg Tyr Asp Trp Gly
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Lys Leu Tyr Ala Ala Thr Ser Phe Tyr Asp Pro Leu Lys Gln Arg Arg
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Ile Met Leu Gly Tyr Val Gly Glu Thr Asp Ser Ala Arg Ala Asp Val
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Ala Lys Gly Trp Ala Ser Leu Gln Ser Ile Pro Arg Thr Val Thr Leu
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Asp Glu Lys Thr Arg Thr Asn Leu Leu Leu Trp Pro Val Glu Glu Val
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           405
Glu Ala Leu Arg Tyr Asn Ser Thr Asp Leu Ser Gly Ile Thr Ile Asp
       420 425
Asn Gly Ser Val Phe His Leu Pro Leu His Gln Ala Thr Gln Leu Asp
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Ile Glu Ala Ser Phe Arg Leu Asp Ala Ser Asp Val Ala Ala Ile Asn
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Glu Ala Asp Val Gly Tyr Asn Cys Ser Ser Ser Gly Gly Ala Ala Ala
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Arg Gly Ala Ile Gly Pro Phe Gly Leu Leu Val His Ala Ala Gly Asp
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Asp Gly Thr Leu Arg Thr Ser Phe Cys Asn Asp Glu Thr Arg Ser Ser
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Arg Ala Arg Asp Val Thr Lys Arg Val Val Gly Ser Thr Val Pro Val
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Leu Asp Gly Glu Ala Leu Ser Met Arg Val Leu Val Asp His Ser Ile
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Val Gln Ser Phe Ala Met Gly Gly Arg Val Thr Ala Thr Ser Arg Val
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Tyr Pro Thr Glu Ala Ile Tyr Ala Arg Ala Gly Val Tyr Leu Phe Asn
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Leu Leu Val Ala Ala Ser Leu Leu Ala Gly Ser Arg Met Gly Gln Ala
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Gly Asp Thr Asp Glu Asp Gly Ala Gly Gly Phe Pro Trp Ser Asn Glu
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                70
Met Leu Gln Trp Gln Arg Ala Gly Phe His Tyr Gln Pro Glu Gly His
                       90
             85 ·
Phe Met Ser Asp Pro Asp Gly Pro Val Tyr Tyr Arg Gly Tyr Tyr His
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                        105
Leu Phe Phe Gln Tyr Asn Arg Arg Gly Val Ala Trp Asp Asp Tyr Ile
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Pro 145	Leu	Ala	Met	Arg	Pro 150	Asp	His	Trp	Tyr	Asp 155	Lys	Lys	Gly	Val	Leu 160
Ser	Gly	Thr	Ile	Thr 165	Val	Leu	His	Asn	Gly 170	Thr	Leu	Val	Leu	Leu 175	Tyr
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			Gly	245					250					255	
			Phe 260					265					270		
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-			Asp	325					330					335	
			Gly 340					345					350		
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			Val 500					505					510		
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545			Met		550					555	i				560
			Gly	565					570	1				575	
			580					585					590		
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Val	610		Glu	Thr	val	615		ьys	ASP	ser	-				

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<210> 70

<211> 422

<212> PRT

<213> Lolium perenne

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<210> 71

<211> 1062

<212> PRT

<213> Festuca arundinacea

<400> 71

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Ala 65	Leu	His	Lys	Thr	Trp 70	Thr	Lys	Val	Val	Ala 75	Met	Arg	Asn	Ser	Gln 80
Glu	Arg	Ser	Asn	Arg 85	Leu	Glu	Asn	Leu	Cys 90	Trp	Arg	Ile	Trp	Asn 95	Val
Ser	Arg	Gln	Lys 100	Lys	Gln	Val	Glu	Trp 105	Asp	Tyr	Thr	Lys	Glu 110	Val	Ala
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145					150					155				Gln	160
				165					170					Arg 175	
		_	180					185					190	Tyr	
		195					200					205		Glu	
	210					215					220			Glu	
225					230					235				Leu	240
				245					250					Glu 255	
			260					265					270	Asp	
		275					280					285		Cys	
	290					295					300			Ile	
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	370					375					380			Leu	
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	450					455					460			Arg	
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Thr	Ala	Glu	Gly	Asp 485		Ala	. Asp	Leu	Gln 490		Leu	Ile	Ala	Pro 495	Asp
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		515					520	Ile	Leu			525	•		Asp
	530	1				535	1				540				Arg
Gln 545		Arg	Glu	Leu	Ala 550		Leu	Thr	Leu	. Ile		. Gly	Asn	Arg	Asp 560

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Leu	Lys	Leu	Ile 580	Asp	Arg	Tyr	Asp	Leu 585	Tyr	Gly	Gln	Val	Ala 590	Tyr	Pro
Lys	His	His 595	Lys	Gln	Thr	Asp	Val 600	Pro	His	Ile	Tyr	Arg 605	Leu	Ala	Ala
Lys	Thr 610		Gly	Val	Phe	Thr 615		Pro	Ala	Leu	Val 620	Glu	Pro	Phe	Gly
625	Thr				630	Ala				635	Pro			Ala	640
Lys	Asn	Gly	Gly	Pro 645	Val	Asp	Ile	Leu	Lys 650	Ala	Leu	Asn	Asn	Gly 655	Leu
Leu	Val	Asp	Pro 660	His	Ser	Ala	Glu	Ala 665	Ile	Thr	Gly	Ala	Leu 670	Leu	Ser
Leu	Leu	Ala 675		Lys	Ser	Arg	Trp 680	Val	Glu	Cys	Arg	Arg 685	Asn	Gly	Leu
Arg	Asn 690		His	Arg	Phe	Ser 695	Trp	Pro	His	His	Cys 700	Arg	Leu	Tyr	Leu
Ser 705	His	Val	Ser	Thr	Tyr 710		Asp	Gln	Pro	Ser 715	Pro	His	Gln	Pro	Leu 720
Arg	Val	Pro	Leu	Gly 725		Gly	Ser	Ser	Thr 730	Ser	Phe	Gly	Ala	Asp 735	Asp
Ser	Leu	Ser	Asp 740		Leu	Arg	Gly	Leu 745	Ser	Leu	Gln	Ile	Ser 750	Val	Asp
Ala	Ser	Ser 755	Asp	Leu	Asn	Ala	Ala 760	Asp	Ser	Ala	Ala	Ala 765	Ile	Met	Asp
	770	Arg	Arg			775					780			Gly	
Arg 785	Ala	Leu	Gly	Phe	Ala 790	Pro	Gly	Arg	Arg	Glu 795	Ser	Leu	Leu	Val	Val 800
	Val	Asp	Cys	Tyr 805	Gly	Asp	Asp	Gly	Lуs 810		Asp	Val	Lys	Gln 815	Leu
Lys	Lys	Ala	Ile 820	Asp	Ala	Ala	Val	Ser 825	Val	Gly	Glu	Cys	Ala 830	Gly	Ala
Lys	Gln	Gly 835		Val	Leu	Ser	Thr 840	Gly	Met	Thr	Ile	Pro 845	Glu	Ala	Ala
Glu	Ala 850	Ile		Ala	Cys	Gly 855	Ala	Asp	Val	Ala	Ser 860	Phe	Asp	Ala	Leu
Ile 865	Cys	Ser	Ser	Gly	Ala 870	Glu	Leu	Cys	Tyr	Pro 875		Lys	Glu	Leu	Ala 880
Ala	Asp	Glu	Glu	Tyr 885		Gly	His	Val	Ala 890		Arg	Trp	Pro	Gly 895	Asp
His	Val	Lys	Ser 900		Val	Pro	Arg	Leu 905	Gly	Ser	Leu	Glu	Glu 910	Ile	Ala
Leu	Ala	Ile 915		Arg	Pro	Ala	Cys 920		Val	His	Cys	His 925		Tyr	Ala
	930		•			935					940			Lys	
945					950					955				Ala	960
				965					970	1				975	
			980	1				985					990		
		995	;				100	0				100	15		Gly
Val	His 101		Thr	Leu	ılle	Leu 101		Gly	Met	: Val	Ala 102		gly	y Ser	Glu
102	Leu !5	Lev			103	Asp 0	Gly			103	5				Ala 1040
Met	Asp	Ser	r Pro	Asn 104	ılle		Thr	Leu	Ala 105	ı Glu		Glr	ser	Ala 105	Ser 5
Asp	Leu	ı Lev	106	. Ala		<u> </u>									

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Pro	Asp	Pro	Pro	Ile 485	Trp	Ala	Asp	Ile	Met 490	Arg	Phe	Phe	Ser	Asn 495	Pro
			500					505					510	Lys	
		515					520					525		Arg	
	530					535					540			Asp	
545					550					555				Leu	560
				565					570					His 575	
			580					585					590	Lys -	
		595					600					605		Leu	
	610					615					620			Gly	
625					630					635				Asp	640
				645					650					Ser 655	
			660					665					670	Ile Val	
_		675					680					685		Ala	
	690					695					700			Val	
705					710					715					720
				725					730					Ala 735	
			740					745					750	Asn	
		755					760					765		Thr	
	770					775					780			Asn	
785					790					795				Ser	800
				805					810					Ala 815	
_	_		820					825					830	Thr Ile	
-		835					840					845		Leu	
	850					855					860			Leu	
865					870					875					880
				885					890	1				Trp 895	
			900					905	i				910		
		915	,				920	ı				925	1	Ser	
	930					935	i				940			Pro	
Val 945	_	Asp	Leu	. Arg	· ьуз 950		Met	Arg	l TTE	955 955		ьeu	Arg	Cys	960
Val	Leu	Туг	Ser	His 965	Asp		Ser	Lys	ь Leu	ı Asn		Ile	Pro	Leu 975	Leu
Ala	Ser	Arg	Ser 980	Gln		. Leu	l Arg	Туг 985	Leu		Ile	Arg	Trp 990	Gly	
Glu	Leu	Ala 995	Asn		Thr	Val	Val	. Val		glu,	Ser	Gly 100	Asp 5	Thr	Asp

Tyr Glu Gly Leu Leu Gly Gly Val His Lys Thr Ile Ile Leu Lys Gly 1015 1020 Ser Phe Asn Ala Ala Pro Asn Gln Leu His Ala Ala Arg Ser Tyr Ser 1025 1030 1035 Leu Glu Asp Val Ile Ser Phe Asp Lys Pro Gly Ile Ala Ser Val Glu 1045 1050 1055 Gly Tyr Leu Pro Asp Ser Leu Lys Ser Ala Leu Gln Gln Phe Gly Val 1060 1065 1070

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Leu Gly Arg Met Pro Gly Pro Glu Ile Gln Gly Thr Tyr Lys Ile Ala 395 3.90 Arg Arg Ile Glu Ala Glu Glu Thr Gly Leu Asp Thr Ala Glu Met Val 405 410 Val Thr Ser Thr Lys Gln Glu Ile Glu Glu Gln Trp Gly Leu Tyr Asp 425 420 Gly Phe Asp Leu Met Val Glu Arg Lys Leu Arg Val Arg Gln Arg Arg 435 440 445 Gly Val Ser Ser Leu Gly Arg Tyr Met Pro Arg Met Ala Val Ile Pro 460 450 455 Pro Gly Met Asp Phe Ser Phe Val Glu Thr Gln Asp Thr Ala Asp Gly 470 475 Thr Gly Arg Ser Gln Met Leu Ile Ala Pro Asp Lys Ala Lys Lys Ala 490 485 Leu Pro Pro Ile Trp Ser Asp Val Leu Arg Phe Phe Thr Asn Pro His 500 505 Lys Pro Met Ile Leu Ala Leu Ser Arg Pro Asp Pro Lys Lys Asn Val 520 525 515 Thr Thr Leu Leu Lys Ala Tyr Gly Glu Ser Arg Gln Leu Arg Glu Leu 540 530 535 Ala Asn Leu Thr Leu Ile Leu Gly Asn Arg Asp Asp Ile Glu Asp Met 550 555 Ala Gly Gly Gly Ala Val Leu Thr Ala Val Leu Lys Leu Ile Asp 565 570 Arg Tyr Asp Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys Gln 580 585 Thr Asp Val Pro His Ile Tyr Arg Leu Ala Ala Lys Thr Lys Gly Val 600 605 Phe Ile Asn Pro Ala Leu Val Glu Pro Phe Gly Leu Thr Ile Ile Glu 620 610 615 Ala Ala Ala Tyr Gly Leu Pro Val Val Ala Thr Lys Asn Gly Gly Pro 625 630 635 Val Asp Ile Leu Lys Ala Leu His Asn Gly Leu Leu Val Asp Pro His 645 650 655 Ser Ala Glu Ala Ile Thr Gly Ala Leu Leu Ser Leu Leu Ala Glu Lys 665 670 660 Ser Arg Trp Val Glu Cys Arg Arg Asn Gly Leu Arg Asn Ile His Arg 680 685 Phe Ser Trp Pro His His Cys Arg Leu Tyr Leu Ser His Val Ser Thr 690 695 700 Tyr Cys Asp Gln Pro Ser Pro His Gln Pro Leu Arg Val Pro Leu Ala 705 710 715 Leu Gly Ser Ser Thr Ser Phe Gly Ala Asp Asp Ser Leu Ser Asp Ser 725 730 735 Leu Arg Gly Leu Ser Leu Gln Ile Ser Val Asp Ala Ser Ser Asp Leu 745 740 Asn Ala Ala Asp Ser Ala Ala Ala Ile Met Asp Ala Leu Arg Arg Arg 765 760 Pro Ala Ser Glu Lys Pro Ala Ser Ser Gly Ala Arg Ala Leu Gly Phe 780 770 775 Ala Pro Gly Arg Arg Glu Ser Leu Leu Val Val Ala Val Asp Cys Tyr 790 795 Gly Asp Asp Gly Lys Pro Asp Val Glu Gln Leu Lys Lys Ala Ile Asp 805 810 Ala Ala Val Ser Val Gly Glu Cys Ala Gly Ala Lys Gln Gly Tyr Val 830 825 Leu Ser Thr Gly Met Thr Ile Pro Glu Ala Ala Glu Ala Ile Lys Ala 845 835 840 Cys Gly Ala Asp Val Ala Ser Phe Asp Ala Leu Ile Cys Ser Ser Gly 850 855 860 Ala Glu Leu Cys Tyr Pro Trp Lys Lys Leu Val Ala Asp Glu Glu Tyr 870 875 Ser Gly His Val Ala Phe Arg Trp Pro Gly Asp His Val Lys Ser Ala 890 895 885 Val Pro Arg Leu Gly Ser Met Glu Glu Ile Ala Leu Ala Ile Asp Arg Pro Ala Ser Ser Val His Cys His Ala Tyr Ala Ala Thr Asp Ala Ser 920 91.5 Lys Val Ser Ile Thr Glu His Tyr Leu <210> 74 <211> 808 <212> PRT <213> Lolium perenne <400> 74 Met Ala Ala Lys Leu Thr Arg Leu His Ser Leu Arg Glu Arg Leu Gly 10 1 5 Ala Thr Phe Ser Ser His Pro Asn Glu Leu Ile Ala Leu Phe Ser Lys 25 20 Tyr Val His Gln Gly Lys Gly Met Leu Gln Arg His Gln Leu Leu Thr 40 Glu Phe Glu Ala Leu Phe Glu Ala Asp Lys Glu Arg Tyr Ala Pro Phe 60 50 55 Glu Asp Ile Leu Arg Ala Ala Gln Glu Ala Ile Val Leu Pro Pro Trp 75 80 65 70 Val Ala Leu Ala Ile Arg Pro Arg Pro Gly Val Trp Asp Tyr Ile Arg 90 85 Val Asn Val Ser Glu Leu Ala Val Glu Glu Leu Thr Val Ser Glu Tyr . 100 105 Leu Ala Phe Lys Glu Gln Leu Val Asp Glu His Ala Ser Ser Lys Phe 125 115 120 Val Leu Glu Leu Asp Phe Glu Pro Phe Asn Ala Ser Phe Pro Arg Pro 130 135 140 Ser Met Ser Lys Ser Ile Gly Asn Gly Val Gln Phe Leu Asn Arg His 145 150 155 Leu Ser Ser Lys Leu Phe Gln Asp Lys Glu Ser Leu Tyr Pro Leu Leu 165 170 175 Asn Phe Leu Lys Ala His Asn His Gln Gly Thr Thr Met Met Leu Asn 185 190 Asp Arg Ile Gln Ser Leu Arg Gly Leu Gln Ser Ala Leu Arg Lys Ala 205 200 195 Glu Glu Tyr Leu Thr Ser Ile Pro Glu Asp Thr Pro Ser Ser Glu Phe 210 215 220 Asn His Arg Phe Gln Glu Leu Gly Leu Glu Lys Gly Trp Gly Asp Thr 230 235 Ala Lys Arg Val Gln Asp Thr Ile His Leu Leu Leu Asp Leu Leu Glu 245 250 Ala Pro Asp Pro Ala Ser Leu Glu Lys Phe Leu Gly Thr Ile Pro Met 270 265 Met Phe Asn Val Val Ile Leu Ser Pro His Gly Tyr Phe Ala Gln Ser 285 280 275 Asn Val Leu Gly Tyr Pro Asp Thr Gly Gly Gln Val Val Tyr Ile Leu 300 290 295 Asp Gln Val Arg Ala Leu Glu Asn Glu Met Leu Leu Arg Ile Lys Gln 310 315 Gln Gly Leu Asp Ile Thr Pro Lys Ile Leu Ile Val Thr Arg Leu Leu 325 330 335 Pro Asp Ala Val Gly Thr Thr Cys Gly Gln Arg Leu Glu Lys Val Ile 345 350 Gly Thr Glu His Thr Asp Ile Leu Arg Val Pro Phe Arg Thr Glu Lys 355 . 360 365 Gly Ile Leu Arg Lys Trp Ile Ser Arg Phe Asp Val Trp Pro Tyr Leu 370 375 380 Glu Thr Tyr Thr Glu Asp Val Ala Asn Glu Leu Met Arg Glu Met Gln 395 390 Thr Lys Pro Asp Leu Ile Ile Gly Asn Tyr Ser Asp Gly Asn Leu Val 405 410 415 Ala Thr Leu Leu Ala His Lys Leu Gly Val Thr Gln Cys Thr Ile Ala 425

PCT/NZ02/00239

55

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His Ala Leu Glu Lys Thr Lys Tyr Pro Asn Ser Asp Ile Tyr Leu Asp
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Lys Phe Asp Ser Gln Tyr His Phe Ser Cys Gln Phe Thr Ala Asp Leu
                  455
                                  460
Ile Ala Met Asn His Thr Asp Phe Ile Ile Thr Ser Thr Phe Gln Glu
                                475
               470
Ile Ala Gly Ser Lys Asp Ser Val Gly Gln Tyr Glu Ser His Ile Ala
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                            490
Phe Thr Leu Pro Asp Leu Tyr Arg Val Val His Gly Ile Asp Val Phe
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Asp Pro Lys Phe Asn Ile Val Ser Pro Gly Ala Asp Met Thr Val Tyr
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                      520
Phe Pro Tyr Thr Glu Thr Asp Lys Arg Leu Thr Ala Phe His Pro Glu
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Ile Glu Glu Leu Leu Tyr Ser Asp Val Glu Asn Ser Glu His Lys Phe
545 550
                                555
Val Leu Lys Asp Lys Asn Lys Pro Ile Ile Phe Ser Met Ala Arg Leu
          565 570 575
Asp Arg Val Lys Asn Met Thr Gly Leu Val Glu Met Phe Gly Lys Asn
         580 585 590
Ala His Leu Lys Asp Leu Ala Asn Leu Val Ile Val Ala Gly Asp His
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Gly Lys Glu Ser Lys Asp Arg Glu Glu Gln Ala Glu Phe Lys Arg Met
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Tyr Ser Leu Ile Glu Glu Tyr Lys Leu Glu Gly His Ile Arg Trp Ile
625 630
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Ser Ala Gln Met Asn Arg Val Arg Asn Ala Glu Leu Tyr Arg Tyr Ile
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Cys Asp Thr Lys Gly Ala Phe Val Gln Pro Ala Phe Tyr Glu Ala Phe
                         665 670
Gly Leu Thr Val Val Glu Ala Met Thr Cys Gly Leu Pro Thr Ile Ala
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 675
Thr Cys His Gly Gly Pro Ala Glu Ile Ile Val Asn Gly Val Ser Gly
  690 695
Leu His Ile Asp Pro Tyr His Ser Asp Lys Ala Ala Asp Ile Leu Val
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               710
Asn Phe Phe Glu Lys Ser Thr Ala Asp Pro Thr Tyr Trp Asp Lys Met
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Ser Glu Gly Gly Leu Lys Arg Ile Tyr Glu Lys Tyr Thr Trp Lys Leu
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Tyr Ser Glu Arg Leu Met Thr Leu Thr Gly Val Tyr Gly Phe Trp Lys
 755 760
                                       765
Tyr Val Ser Asn Leu Glu Arg Arg Glu Thr Arg Arg Tyr Leu Glu Met
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Phe Tyr Ala Leu Lys Tyr Arg Ser Leu Ala Ala Ala Val Pro Leu Ala
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Val Asp Gly Glu Asn Thr Asp Asn
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          20
                          25
Tyr Val His Gln Gly Lys Gly Met Leu Gln Arg His Gln Leu Leu Thr
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40 Glu Phe Glu Ala Leu Phe Glu Ser Asp Lys Glu Arg Tyr Ala Pro Phe

Gln Asp Ile Leu Arg Ala Ala Gln Glu Ala Ile Val Leu Pro Pro Trp

60

5.5

Val	Ala	Leu	Ala	Ile 85	Arg	Pro	Arg	Pro	Gly 90	Val	Trp	Asp	Tyr	Ile 95	Arg
Val	Asn	Val	Ser 100	Glu	Leu	Ala	Val	Glu 105	Glu	Leu	Thr	Val	Ser 110	Glu	Tyr
Leu	Ala	Phe 115	Lys	Glu	Gln	Leu	Val 120	Asp	Glu	His	Ala	Ser 125	Ser	Lys	Phe
Val	Leu 130	Glu	Leu	Asp	Phe'	Glu 135		Phe	Asn	Ala	Ser 140	Phe	Pro	Arg	Pro
Ser 145		Ser	Lys	Ser	Ile 150		Asn	Gly	Val	Gln 155	Phe	Leu	Asn	Arg	His 160
	Ser	Ser	Lys	Leu 165	Phe	Gln	Asp	Lys	Glu 170	Ser	Leu	Tyr	Pro	Leu 175	Leu
Asn	Phe	Leu	Lys 180	Ala	His	Asn	His	Lys 185	Gly	Thr	Thr	Met	Met 190	Leu	Asn
		Ile 195					200					205			
	210	Tyr				215					220				
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	_	Arg		245	_				250					255	
		Asp	260					265					270		
		Asn 275					280					285			
	290	Leu				295					300				
305		Val			310					315					320
		Leu		325					330					335	
		Ala	340					345					350		
_		Glu 355					360					365			
_	370	Leu -				375					380				
385		Tyr			390					395					400
		Pro		405					410					415	
		Leu	420			-		425					430		
		Leu 435					440					445			
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465		Met			470					475					480
		Gly		485					490					495	
		Leu	500					505					510		
		Lys 515					520		•			525			
	530	Tyr				535					540				
545		Glu -			550					555					560
		Lys		565		_		•	570					575	
		Val	580					585					590		
ALa	HIS	Leu 595	туѕ	Asp	ьeu	ATa	Asn 600		Val	Ile	val	A1a 605		Asp	nlS

Gly Lys Glu Ser Lys Asp Arg Glu Glu Gln Ala Glu Phe Lys Arg Met 620 615 610 Tyr Ser Leu Ile Glu Glu Tyr Lys Leu Lys Gly His Ile Arg Trp Ile 630 635 Ser Ala Gln Met Asn Arg Val Arg Asn Ala Glu Leu Tyr Arg Tyr Ile 650 655 645 Cys Asp Thr Lys Gly Ala Phe Val Gln Pro Ala Phe Tyr Glu Ala Phe 660 665 670 Gly Leu Thr Val Ile Glu Ala Met Thr Cys Gly Leu Pro Thr Ile Ala 675 . 680 685 Thr Cys His Gly Gly Pro Ala Glu Ile Ile Val Asp Gly Val Ser Gly 700 690 695 Leu His Ile Asp Pro Tyr His Ser Asp Lys Ala Ala Asp Ile Leu Val 710 715 Asn Phe Phe Glu Lys Ser Thr Ala Asp Pro Thr Tyr Trp Asp Lys Met 735 725 730 Ser Glu Gly Gly Leu Lys Arg Ile Tyr Glu Lys Tyr Thr Trp Lys Leu 740 745 Tyr Ser Glu Arg Leu Met Thr Leu Thr Gly Val Tyr Gly Phe Trp Lys 765 755 760 Tyr Val Ser Asn Leu Glu Arg Arg Glu Thr Arg Arg Tyr Leu Glu Met 775 780 Phe Tyr Ala Leu Lys Tyr Arg Ser Leu Ala Ala Ala Val Pro Leu Ala 785 790 795 Val Asp Gly Glu Asn Thr Asp Ser 805 .

<210> 76

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<212> PRT

<213> Lolium perenne

<400> 76

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Leu Leu Asp Leu Leu Glu Ala Pro Asp Pro Ser Thr Leu Glu Lys 2.60 265 Phe Leu Gly Thr Ile Pro Met Val Phe Asn Val Val Ile Leu Ser Pro 280 285 His Gly Tyr Phe Ala Gln Ala Asn Val Leu Gly Tyr Pro Asp Thr Gly 300 295 Gly Gln Val Val Tyr Ile Leu Asp Gln Val Arg Ala Met Glu Asn Glu 305 310 315 320 Met Leu Leu Arg Ile Lys Gln Gln Gly Leu Asp Ile Thr Pro Arg Ile 325 330 335 Gln Arg Leu Glu Lys Val Leu Gly Thr Glu His Thr His Ile Leu Arg 355 360 Val Pro Phe Arg Thr Glu Asn Gly Ile Val Arg Lys Trp Ile Ser Arg 370 375 380 Phe Glu Val Trp Pro Tyr Leu Glu Thr Phe Thr Asp Asp Val Ala His 390 395 Glu Ile Ser Gly Glu Leu Gln Ala Asn Pro Asp Leu Ile Ile Gly Asn 405 410 415 Tyr Ser Asp Gly Asn Leu Val Ala Cys Leu Leu Ala His Lys Met Gly 420 425 430 Val Thr His Cys Thr Ile Ala His Ala Leu Glu Lys Thr Lys Tyr Pro 435 440 Asn Ser Asp Leu Tyr Trp Lys Lys Phe Glu Asp His Tyr His Phe Ser 450 455 460 Cys Gln Phe Thr Thr Asp Leu Ile Ala Met Asn His Ala Asp Phe Ile 475 480 470 Ile Thr Ser Thr Phe Gln Glu Ile Ala Gly Asn Lys Asp Thr Val Gly 485 490 495 Gln Tyr Glu Ser His Met Ala Phe Thr Met Pro Gly Met Tyr Arg Val 500 505 510 Val His Gly Ile Asp Val Phe Asp Pro Lys Phe Asn Ile Val Ser Pro 515 520 525 Gly Ala Asp Met Ser Ile Tyr Phe Pro Tyr Ser Glu Ser Gln Arg Arg 530 535 540 Leu Thr Ser Leu His Pro Glu Ile Glu Glu Leu Leu Tyr Ser Asp Val 550 555 Asp Asn Asp Glu His Ser Cys Leu Lys Asp Arg Asn Lys Pro Ile Ile 565 570 575 Phe Ser Met Ala Arg Leu Asp Arg Val Lys Asn Leu Thr Gly Leu Val 580 585 590 Glu Leu Tyr Gly Arg Asn Pro Arg Leu Gln Glu Leu Val Asn Leu Val 595 600 605 Val Val Cys Gly Asp His Gly Asn Pro Ser Lys Asp Lys Glu Glu Gln 615 620 Ala Glu Phe Lys Lys Met Phe Asp Leu Ile Glu Gln Tyr Asn Leu Asn 635 630 Gly His Ile Arg Trp Ile Ser Ala Gln Met Asn Arg Val Arg Asn Ala 645 650 655 Glu Leu Tyr Arg Tyr Ile Cys Asp Thr Lys Gly Ala Phe Val Gln Pro 660 665 670 Ala Phe Tyr Glu Ala Phe Gly Leu Thr Val Ile Glu Ala Met Thr Cys 675 680 Gly Leu Pro Thr Phe Ala Thr Ala Tyr Gly Gly Pro Ala Glu Ile Ile 695 700 Val Asn Gly Val Ser Gly Tyr His Ile Asp Pro Tyr Gln Gly Asp Lys 710 715 Ala Ser Ala Leu Leu Val Glu Phe Phe Glu Lys Cys Gln Gly Asp His 725 730 735 Ser His Trp Thr Lys Ile Ser Leu Gly Gly Leu Gln Arg Ile Glu Glu 740 745 Lys Tyr Thr Trp Lys Leu Tyr Ser Glu Arg Leu Met Thr Leu Thr Gly 755 760 765 Val Tyr Gly Phe Trp Lys Tyr Val Ser Asn Leu Glu Arg Arg Glu Thr 775

 Arg Arg Tyr Leu Glu Met Leu Tyr Ala Leu Lys Tyr Arg Thr Met Ala

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 790
 795
 800

 Ser Thr Val Pro Leu Ala Val Glu Gly Glu Pro Ser Ser Lys
 805
 810

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<213> Festuca arundinacea

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375

420

Asn Tyr Ser Asp Gly Asn Leu Val Ala Cys Leu Leu Ala His Lys Leu

425

405 410 415

Gly Val Thr His Cys Thr Ile Ala His Ala Leu Glu Lys Thr Lys Tyr 440 Pro Asn Ser Asp Leu Tyr Trp Lys Lys Phe Glu Asp His Tyr His Phe 455 460 Ser Cys Gln Phe Thr Ala Asp Leu Ile Ala Met Asn His Ala Asp Phe 475 465 470 Ile Ile Thr Ser Thr Phe Gln Glu Ile Ala Gly Asn Lys Asp Thr Val 485 490 495 Gly Gln Tyr Glu Ser His Met Ala Phe Thr Met Pro Gly Leu Tyr Arg 500 505 510 Val Val His Gly Ile Asp Val Phe Asp Pro Lys Phe Asn Ile Val Ser 525 520 Pro Gly Ala Asp Met Thr Ile Tyr Phe Pro Tyr Thr Glu Gln Gln Lys 535 540 Arg Leu Thr Ser Leu His Ala Glu Ile Glu Glu Leu Leu Phe Ser Asp 545 550 555 Val Glu Asn Ser Glu His Lys Phe Val Leu Lys Asp Lys Asn Lys Pro · 565 570 575 Ile Ile Phe Ser Met Ala Arg Leu Asp Arg Val Lys Asn Met Thr Gly 585 590 Leu Val Glu Leu Tyr Gly Arg Asn Pro Arg Leu Gln Glu Leu Val Asn 605 595 600 Leu Val Val Val Cys Gly Asp His Gly Lys Glu Ser Lys Asp Lys Glu 610 615 620 Glu Gln Ala Glu Phe Lys Arg Met Phe Asp Leu Ile Glu Gln Tyr Asn 635 630 Leu Ser Ser His Ile Arg Trp Ile Ser Ala Gln Met Asn Arg Val Arg 650 655 645 Asn Gly Glu Leu Tyr Arg Tyr Ile Cys Asp Met Lys Gly Ala Phe Val 660 665 670 Gln Pro Ala Phe Tyr Glu Ala Phe Gly Leu Thr Val Ile Glu Ala Met 685 675 680 Thr Cys Gly Leu Pro Thr Phe Ala Thr Ala Tyr Gly Gly Pro Ala Glu 690 695 700 Ile Ile Val Asn Gly Val Ser Gly Tyr His Ile Asp Pro Tyr Gln Asn 710 . 715 Asp Lys Ala Ser Ala Leu Leu Val Glu Phe Phe Glu Lys Cys Gln Glu 730 735 725 Asp Pro Ser His Trp Asn Lys Ile Ser Gln Gly Gly Leu Gln Arg Ile 740 745 750 Glu Glu Lys Tyr Thr Trp Lys Leu Tyr Ser Glu Arg Leu Met Thr Leu 755 760 765 Ser Gly Val Tyr Gly Phe Trp Lys Tyr Val Ser Asn Leu Asp Arg Arg 770 775 780 Glu Thr Arg Arg Tyr Leu Glu Met Leu Tyr Ala Leu Lys Tyr Arg Lys 795 790 Met Ala Thr Thr Val Pro Leu Ala Ile Glu Gly Glu Thr Thr Gly Lys 810 815 <210> 78 <211> 478 <212> PRT <213> Festuca arundinacea

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Leu Leu Tyr Lys Ser Glu Asp Phe Leu Asn Trp Ser Arg Val Asp His
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115 120
Pro Leu Tyr Ser Ser Ser Ala Ser Thr Met Trp Glu Cys Leu Asp Phe
 130 135 140
Phe Ala Val Leu Pro Gly Ser Asn Gly Gly Leu Asp Leu Ser Ala Ala
145 150 155
Ile Pro Lys Gly Ala Lys His Val Leu Lys Val Ser Val Asp Gln Cys 165 170 170
Asp Lys Tyr Met Ile Gly Val Tyr Asp Leu Glu His Asp Ala Phe Val
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Pro Asp Thr Ile Leu Asp Asp Arg Trp Leu Leu Pro Arg Ile Asp Tyr
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Gly Asn Tyr Tyr Ala Ser Lys Ser Phe Phe Asp Ser Lys Asn Arg Arg
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Val Ala Lys Gly Trp Ala Gly Ile Tyr Ala Ile Pro Arg Thr Ile Trp 245 250 255
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Leu Asp Arg Asp Gly Lys Gln Leu Leu Gln Trp Pro Val Glu Glu Ile
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Glu Ser Leu Arg Arg Asn Glu Ile Asn Tyr Gln Gly Leu Asp Leu Glu
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Lys Gly Asp Leu Tyr Glu Ile Lys Gly Val Asp Thr Leu Gln Ala Asp
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Val Glu Ile Asp Phe Glu Leu Thr Ser Ile Asp Asp Ala Asp Ser Phe
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Asp Pro Ser Trp Leu Leu Asp Pro Glu Lys His Cys Arg Glu Ala Gly
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Ala Ser Val His Gly Gly Ile Gly Pro Phe Gly Leu Val Ile Leu Ala
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Thr Gly Asp Met Glu Glu His Thr Val Val His Phe Arg Val Tyr Lys
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Ser Gln Lys Glu Tyr Met Ile Leu Met Cys Ser Asp Ile Arg Arg Ser
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Ser Leu Arg Gln Gly Leu Tyr Ala Pro Ala Tyr Gly Gly Phe Phe Glu
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Phe Asp Leu Glu Lys Glu Arg Lys Ile Ser Leu Arg Thr Leu Ile Asp 405 410 415
Arg Ser Ala Val Glu Ser Phe Gly Gly Gly Arg Val Cys Ile Ile
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Ser Ala Ala Ser Pro Ser Val Pro Ala Ser Ile Val Ser Pro Leu Leu 35 40 45

Arg Thr Gly Tyr His Phe Gln Pro Pro Met Asn Trp Ile Asn Asp Pro

Thr I le	His Ser Val 95 Ile Ser Pro 110 Thr Thr Ile Asp Arg Pro Asn Ala Ser 160 Pro Ile Ala 175 Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240
Ser Arg Asp Leu Ile Asp Icu Ile Asp Icu Ile Asp Irp Ile Ala Leu Ile Asp Glu Trp Ile Ala Leu Ile Pro Thr Asp Glu Tyr Glu Ile Leu Tyr Glu Ile Leu Tyr Glu Ile Leu Tyr Ile Asp Ile Asp Ile Ile <td>95 Ile Ser Pro 110 Thr Thr Ile Asp Arg Pro Asn Ala Ser 160 Pro Ile Ala 175 Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu</td>	95 Ile Ser Pro 110 Thr Thr Ile Asp Arg Pro Asn Ala Ser 160 Pro Ile Ala 175 Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
The leaf of the le	Thr Thr Ile Asp Arg Pro Asn Ala Ser 160 Pro Ile Ala 175 Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
115	Asp Arg Pro Asn Ala Ser 160 Pro Ile Ala 175 Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
Ser Val Asn Tyr Gln Ile Gln Asn Ile Ala Leu Pro Lys Asn 145	Asn Ala Ser 160 Pro Ile Ala 175 Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
145 Asp Pro Leu Leu Arg Glu Trp Tyr Lys Pro Gly Tyr Asp Pro Gly Tyr Asp Pro Gly Tyr Asp Pro Gly Tyr Asp Pro 170 Tyr Asp Pro 185 Tyr Asp Asp Pro 200 Tyr Arg Asp Pro Asp P	Pro Ile Ala 175 Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
Asp Pro Leu Leu Arg Glu Trp Tyr Lys Pro Gly Tyr Asp Pro Hys Pro Asp Asp Pro Val Pro Val Gly Ile Asp Ala Thr Gln Phe Arg Pro 195 Ala Pro Gly Thr Leu Arg Gly Arg	Pro Thr Thr 190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
Ala Trp Phe Ala Gly Arg His Trp Arg Met Leu Val Gly Gl Gl Gl Arg Met Leu Val Gly Gl Gl Arg Arg Met Leu Val Gly Gl Arg Arg <td>190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu</td>	190 Gly Leu Arg Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
Ala Trp Phe Ala Gly Arg His Trp Arg Met Leu Val Gly Cly Arg Arg Interpretation Arg Interpretation Arg Interpretation	Arg Asp Phe Leu Thr Gly 240 Gly Val Glu
Lys His Trp Val Arg Ala Lys His Pro Leu His Ser Ala Lec	Leu Thr Gly 240 Gly Val Glu
Lys His Trp Val Arg Ala Lys His Pro Leu His Ser Ala Lec 225	240 Gly Val Glu
Lys Gly Leu Asp Thr Ser Glu Tyr Gly Ala Ala Ala Gly Va 265 His Val Leu Lys Asn Ser Leu Asp Leu Thr Arg Tyr Asp Tyr Asp Tyr 285 Ile Gly Thr Tyr Asp Asn Val Lys Glu Arg Tyr Val Pro As 290 Thr Gly Asp Val Tyr Gln Arg Leu Gln Tyr Asp Tyr Gly As 315 Ala Ser Lys Thr Phe Phe Asp Pro Val Lys Gln Arg Arg It Asp It Asp Gly Trp Ala Asn Glu Ser Asp Ser Val Ala His Asp Lys Ala Ser Asp Asp Glu Arg It Asp Ser Val Ala His Asp Lys Ala Ser Asp Asp Ser Val Ala His Asp Lys Ala Ser Asp	Gly Val Glu 255
His Val Leu Lys Asn Ser Leu Asp Leu Thr Arg Tyr Asp Tyr 285	
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290	
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355 360 365	350
Gly Lys Gln Leu Val Gln Trp Pro Val Glu Glu Leu Glu Ly	
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Gly Lys Pro Val Asn Val Gly Asp Lys Val Val Lys Pro Gl 385 390 395	400
Phe Glu Val Thr Gly Leu Gln Ser Tyr Gln Ser Asp Val Gl 405 410	415
420	430
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Lys Pro Ile Val Leu Met Cys Ser Asp Pro Thr Lys Ser Se 485 490	495
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Ser Ser Gly Lys Ile Ser Leu Arg Ser Leu Ile Asp Arg Se 515 520 525	
Glu Ser Phe Gly Ala Gly Gly Lys Thr Cys Ile Leu Ser A: 530 535 540	
Pro Ser Met Ala Leu Gly Lys Asp Ala His Leu His Val Pl 545 550 555	560
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PCT/NZ02/00239 63

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405 410 415 Pro Ser Leu Glu Gly Ala Glu Asn Leu Asp Pro Asn Gln Leu Leu Asp 420 425 Pro Gln Arg Leu Cys Gly Glu Lys Gly Ala Ser Val Leu Gly Gly Val 435 440 Gly Pro Phe Gly Leu Leu Val Leu Ala Ser Gly Asp Leu Gln Glu His 460 455 Thr Ser Val Phe Phe Arg Val Phe Arg His Glu Gly Lys Tyr Lys Val 475 470 Leu Met Cys Thr Asp Leu Arg Arg Ser Thr Thr Arg Ala Asp Val Tyr 485 490 495 Lys Pro Pro Tyr Gly Gly Phe Val Asp Ile Asp Ile Glu Lys Glu Arg 500 505 510 Ser Ile Ser Leu Arg Thr Leu Val Asp His Ser Val Val Glu Ser Tyr 515 520 525 Gly Gly Gly Arg Thr Val Ile Thr Ala Arg Val Tyr Pro Glu His 540 535

Ala Ala Thr Thr Asn Ser Arg Leu Phe Met Phe Asn Asn Gly Thr Gly 555 550 Ala Val Lys Val Ser Lys Leu Asp Ala Trp Glu Leu Ala Pro Ala Lys 570 565 Val Asn Val Pro Gly Asp Gly Leu Ile Thr Ala Gly Ser Ser Met His 590 585 580 Leu Arg Glu Ala Tyr 595 <210> 82 <211> 399 <212> PRT <213> Festuca arundinacea <400> 82 Leu Asn Ser Thr Glu Phe Arg Asp Pro Thr Thr Gly Trp Ile Gly Pro 5 10 Asp Gly Leu Trp Arg Ile Ala Ile Gly Ala Glu Leu Asn Gly Tyr Gly 25 Ala Ala Leu Leu Tyr Lys Ser Glu Asp Phe Leu Asn Trp Thr Arg Val 40 35 Asp His Pro Leu Tyr Ser Asp Asn Ala Pro Ser Met Trp Glu Cys Pro 50 55 60 Asp Phe Phe Ala Val Leu Pro Gly Asn Asn Gly Gly Leu Asp Leu Ser 7.5 70 Ala Ala Ile Pro Lys Gly Ala Lys His Val Leu Lys Met Ser Val Asp 90 Tyr Ser Asp Lys Tyr Met Ile Gly Val Tyr Asp Leu Lys Arg Asp Ala 105 110 100 Phe Val Pro Asp Val Val Leu Asp Asp Arg Arg Leu Trp Leu Arg Ile 115 120 125 Asp Tyr Gly Thr Phe Tyr Ala Ser Lys Ser Phe Phe Asp Ser Lys Arg 130 135 140 Gly Arg Arg Val Ile Trp Gly Trp Ser Asn Glu Thr Asp Ser Val Ser 155 160 150 Asp Asp Gly Ala Lys Gly Trp Ala Gly Ile His Ala Ile Pro Arg Ser 170 175 165 Ile Trp Leu Asp Ser Asp Gly Lys Gln Leu Leu Gln Trp Pro Ile Asp 180 185 Glu Ile Glu Ser Leu Arg Arg Asp Glu Ile Asn His Gln Gly Leu Glu 195 200 Leu Lys Asn Gly Asp Leu Phe Glu Ile Lys Gly Ile Asp Thr Leu Gln 220 215 Ala Asp Ile Glu Val Asp Phe Glu Leu Thr Ser Ile Asp Ser Ala Asp 230 235 Pro Phe Asp Pro Ser Trp Leu Leu Asp Val Glu Arg His Cys Arg Glu 250 245 Ala Gly Ala Ser Val Gln Gly Gly Ile Gly Pro Phe Gly Leu Val Val 265 260 Leu Ala Ser Asp Asn Met Glu Glu His Ile Ala Val His Phe Arg Val 275 280 285 Tyr Lys Ser Gln Lys Ser His Met Ile Leu Met Cys Ser Asp Leu Arg 300 295 Arg Ser Ser Leu Arg Ser Gly Leu Tyr Thr Pro Ala Tyr Gly Gly Phe 315 310 Phe Glu Phe Asp Leu Glu Lys Glu Arg Lys Ile Ser Leu Arg Thr Leu 330 325 Ile Asp Arg Ser Ala Val Glu Ser Phe Gly Gly Gly Arg Val Cys 345 340 Ile Thr Ala Arg Ile Tyr Pro Val Ala Leu Val Asp Gly Arg Val His 355 360 365 Met Tyr Ala Phe Asn Asn Gly Ser Thr Thr Val Arg Val Pro Gln Leu 370 375 380 370 375 Gly Ala Trp Ser Met Met Thr Ala Gln Val Asn Val Asn Lys Gly

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Lys Pro Ile Val Leu Met Cys Ser Asp Pro Thr Lys Ser Ser Leu Thr 490 Pro Asp Leu Tyr Lys Pro Thr Phe Ala Gly Phe Val Asp Thr Asp Ile 500 505 Ser Ser Gly Lys Ile Ser Leu Arg Ser Leu Ile Asp Arg Ser Val Val 520 525 515 Glu Ser Phe Gly Ala Gly Gly Lys Thr Cys Ile Leu Ser Arg Val Tyr 535 540 Pro Ser Met Ala Leu Gly Lys Asn Ala His Leu His Val Phe Asn Asn 545 550 555 560 Gly Glu Thr Asp Ile Lys Val Ser Lys Leu Thr Val Trp Glu Met Lys 570 575 565 Arg Pro Leu Met Asn Gly Ala 580 <210> 84 <211> 346 <212> PRT <213> Lolium perenne <400> 84 Met Tyr Tyr Asn Gly Ile Tyr His Glu Phe Tyr Gln Tyr Asn Pro Asn 5 10 Gly Ser Leu Trp Gly Asn Ile Ile Trp Gly His Ser Val Ser Thr Asp Leu Ile Asn Trp Ile Pro Val Glu Pro Ala Ile Glu Arg Asp Ile Pro 40 35 Ser Asp Ile Asn Gly Cys Trp Thr Gly Ser Ala Thr Ile Ile Ser Gly 55 60 Asp Gln Pro Ile Ile Ile Tyr Thr Gly Ala Asp Lys Glu Asn Arg Gln 70 75 Leu Gln Asn Ile Val Leu Pro Lys Asn Lys Ser Asp Pro Tyr Leu Arg 85 90 Glu Trp Thr Lys Ala Gly Asn Asn Pro Val Ile Gln Pro Val Gly Pro 100 105 Gly Leu Asn Ala Ser Gln Phe Arg Asp Pro Thr Thr Gly Trp Ile Gly 120 125 Pro Asp Gly Leu Trp Arg Ile Ala Val Gly Ala Glu Leu Asn Gly Tyr 135 140 Gly Ala Ala Leu Leu Tyr Lys Ser Gln Asp Phe Leu Asn Trp Thr Arg 145 150 155 160 Val Asp His Pro Leu Tyr Ser Ser Asn Ala Ser Ser Met Trp Glu Cys 165 170 Pro Asp Phe Phe Ala Val Leu Pro Gly Asn Ser Gly Gly Leu Asp Leu 185 190 180 Ser Ala Glu Ile Pro Asn Gly Ala Lys His Val Leu Lys Met Ser Leu 200 205 Asp Ser Cys Asp Lys Tyr Met Ile Gly Val Tyr Asp Leu Lys Ser Asp 215 220 Thr Phe Met Pro Asp Ser Val Leu Asp Asp Arg Arg Leu Trp Ser Arg 225 230 235 240 Ile Asp His Gly Asn Phe Tyr Ala Ser Lys Ser Phe Phe Asp Ser Lys 245 250 255 Lys Gly Arg Arg Ile Ile Trp Gly Trp Thr Asn Glu Thr Asp Ser Ser 260 265 270 Ser Asp Asp Val Ala Lys Gly Trp Ala Gly Ile His Ala Ile Pro Arg 280 285 Thr Ile Trp Leu Asp Ser Tyr Gly Lys Gln Leu Leu Gln Trp Pro Ile 295 300 Glu Glu Ile Glu Ser Leu Arg Arg Asn Glu Ile Ser His Gln Gly Leu 315 310 Glu Leu Lys Lys Gly Asp Leu Phe Glu Ile Lys Gly Thr Asp Thr Ser 325 330 Gln Val Val His Val Phe Leu Gly Lys Leu

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115 120 125 115 Gln Pro Ala Ile Leu Tyr Thr Gly Ile Asp Ala Ala Gly Asn Gln Val 130 135 140 Gln Asn Val Ala Phe Pro Lys Lys Ala Ser Asp Pro Leu Leu Arg Glu 145 150 155 Trp Val Lys Pro Asp Tyr Asn Pro Val Ile Pro Leu Pro Lys Asp Val 170 175 165 Val His Asp Ser Phe Arg Asp Pro Ser Thr Ala Trp Arg Gly Arg Asp 180 185 190 Gly Leu Trp Arg Val Ala Ile Ala Ala Lys Val Asn Val Thr Val Thr 195 200 Val Gly Ser Thr Leu Ile Tyr Arg Ser Lys Asp Phe Arg Arg Trp Glu 215 220 Arg Asn Ala Ala Pro Leu Tyr Glu Ser Leu Ala Ala Gly Met Val Glu 225 230 235 Cys Pro Asp Leu Phe Pro Val Ala Lys Pro Gly Ala Gln Asn Gly Leu 250 255 245 Asp Tyr Ala Pro Ser Ser Arg Ala Ala Arg His Val Leu Lys Leu Ser 260 265 Val Val Ala Thr Leu Gln Asp Tyr Tyr Val Val Gly Leu Tyr Asp Asp 275 280 Thr Ala Asp Thr Phe Asn Ala Ala Gly Ala Asp Asn Asp Trp Arg 300 295 Thr Trp Arg Arg Ile Asp Tyr Gly His Val Tyr Ala Ser Lys Ser Phe 310 315 Phe Asp Ala Arg Lys Asn Arg Arg Val Leu Trp Cys Trp Ala Asn Glu 335 330 325 Ser Asp Thr Glu Ala Asp Tyr Ile Ala Arg Gly Trp Ser Gly Val Gln 340 345 350 Thr Val Pro Arg Lys Ile Trp Leu Asp Ile Asp Gly Lys Gln Leu Leu 355 360 365 Gln Trp Pro Ile Lys Glu Ile Glu Thr Leu Arg Lys Lys Arg Val Gly 380 375 Leu Leu Gly Thr Glu Met Asn Ser Gly Gly Leu Asn Glu Ile Ile Gly 390 395 Val Ala Gly Ser Gln Ala Asp Val Glu Val Val Phe Lys Ile Pro Thr 415 410 405 Leu Glu Gly Ala Glu Asn Ile Glu Pro Asn Glu Leu Leu Asp Pro Gln 430 420 425 Lys Leu Cys Gly Asn Asn Gly Ala Ser Met Arg Gly Ser Ile Gly Pro 445 435 440 Phe Gly Leu Leu Leu Ala Ser Gly Asp Leu Leu Glu His Thr Ser 460 455 Val Phe Phe Arg Val Phe Lys His Gly Ala Lys Tyr Lys Val Leu Met

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Cys Thr Asp Leu Thr Arg Ser Thr Thr Arg Ser Asp Val Tyr Lys Pro 490 485 Ser Tyr Gly Gly Phe Val Asp Met Asp Ile Asp Lys Thr Lys Ser Ile 505 510 Ser Leu Arg Thr Leu Ile Asp His Ser Val Val Glu Ser Phe Gly Gly 525 520 515 Gly Gly Arg Thr Cys Ile Thr Ala Arg Val Tyr Pro Glu His Ala Glu 535 540 Met Ser Asn Ser His Ile Tyr Met Phe Asn Asn Gly Thr Gly Ala Val 545 550 555 560 Lys Val Ala Lys Leu Glu Ala Trp Glu Leu Ala Thr Ala Asn Val Asn 570 Val Ala Gly His Gly

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<213> Lolium perenne

<400> 86

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Ala Gly Ile Gln Ala Ile Pro Arg Lys Val Trp Leu Asp Pro Ser Gly 360 Arg Gln Leu Met Gln Trp Pro Val Glu Glu Val Lys Ala Leu Arg Gly 375 380 Lys Lys Pro Val Ser Leu Lys Asp Arg Met Val Lys Arg Gly Glu His 390 395 Val Glu Val Thr Gly Leu Gln Thr Ala Gln Ala Asp Val Glu Val Ser 410 415 405 Phe Glu Val Pro Ser Leu Glu Gly Ala Glu Ala Leu Asp Pro Ala Leu 420 425 430 Ala Asn Asp Ala Gln Lys Leu Cys Gly Val Lys Gly Ala Asp Val Glu 435 440 445 Gly Gly Val Gly Pro Phe Gly Leu Trp Val Leu Ala Ser Ser Lys Leu 450 455 Glu Glu Arg Thr Ala Val Phe Phe Arg Val Phe Lys Ala Ala Gly Asn 475 470 Val Asn Ser Thr Lys Pro Leu Val Leu Met Cys Ser Asp Pro Thr Lys 490 495 485 Ser Ser Leu Asn Lys Asn Leu Tyr His Pro Thr Phe Ala Gly Phe Val 505 510 500 Asp Ile Asp Met Ala Lys Gly Lys Ile Ser Leu Arg Ser Leu Ile Asp 515 520 525 Gln Ser Val Val Glu Ser Phe Gly Ala Gly Gly Arg Thr Cys Ile Leu 530 535 Ser Arg Val Tyr Pro Ser Leu Ala Ile Gly Arg Asn Ala His Leu His 550 555 Val Phe Asn Asn Gly Lys Ala Asp Ile Lys Val Ser Arg Leu Thr Ala 570 575 565 Trp Glu Met Lys Lys Pro Ala Leu Met Asn Gly Ala 580

<210> 87

<211> 668

<212> PRT

<213> Lolium perenne

<400> 87

Met Glu Ala Arg Asp Gly Val Ser Met Pro Tyr Ser Tyr Ala Ala Leu 10 Pro Glu Asp Ala Glu Ala Ala Val Val Gly Arg Gly Arg Thr Gly 25 Pro Leu Phe Ala Ala Leu Leu Leu Thr Leu Val Ala Ala Leu Leu Ala 35 Val Ala Ala Leu Ala Gly Val Arg Leu Val Gly Glu Leu Pro Ala Gly 55 Gly Val Val Met Pro Asn His Pro Met Glu Val Met Asp Val Ser Gly 75 70 Ser Arg Gly Pro Glu Ser Gly Val Ser Glu Lys Thr Ser Gly Ala Ala 90 Ser Glu Ser Gly Gly Met Leu Gly Ala Asp Ala Gly Ser Asn Ala Phe 105 110 100 Pro Trp Ser Asn Ala Met Leu Gln Trp Gln Arg Thr Gly Phe His Phe 115 125 120 Gln Pro Glu Lys Asn Trp Met Asn Asp Pro Asn Gly Pro Val Tyr Tyr 135 140 Lys Gly Trp Tyr His Leu Phe Tyr Gln Tyr Asn Pro Glu Gly Ala Ile 155 150 Trp Gly Asn Lys Ile Ala Trp Gly His Ala Val Ser Arg Asp Met Leu 170 165 Arg Trp Arg His Leu Pro Ile Ala Met Phe Pro Asp Gln Trp Tyr Asp 190 180 185 Ile Asn Gly Ala Trp Ser Gly Ser Ala Thr Val Leu Pro Asp Gly Arg 195 200 205 Ile Val Met Leu Tyr Thr Gly Ser Thr Asn Ala Ser Val Gln Val Gln 215

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Cys Leu Ala Phe Pro Ser Asp Pro Ser Asp Pro Leu Leu Thr Asn Trp 230 235 Thr Lys Tyr Glu Gly Asn Pro Val Leu Tyr Pro Pro Pro His Val Gly 250 255 245 Glu Lys Asp Phe Arg Asp Pro Thr Thr Ala Trp Tyr Asp Gly Ser Asp 270 265 260 Gly Met Trp Arg Ile Val Ile Gly Ser Lys Asp Asn Arg Arg Ala Gly 280 285 275 Met Ala Leu Thr Tyr Lys Thr Lys Asn Phe His Asp Phe Glu Leu Val 300 295 Pro Gly Val Leu His Arg Val Pro Ala Thr Gly Met Trp Glu Cys Ile 310 315 Asp Leu Tyr Pro Val Gly Gly Ala Arg Gly Ile Asp Met Thr Glu Ala 330 325 . Val Ala Ala Ala Ser Asn Ser Gly Gly Gly Glu Val Leu His Val Met 345 350 340 Lys Glu Ser Ser Asp Asp Asp Arg His Asp Tyr Tyr Ala Leu Gly Arg 365 360 355 Tyr Asp Ala Ala Thr Asn Lys Trp Thr Pro Leu Asp Ala Asp Ala Asp 370 375 380 370 375 Val Gly Ile Gly Leu Arg Tyr Asp Trp Gly Lys Phe Tyr Ala Ser Lys 385 390 395 400 Thr Phe Tyr Asp Pro Ala Lys Lys Arg Arg Val Leu Trp Gly Trp Val 405 410 Gly Glu Thr Asp Ser Glu Arg Ala Asp Val Ala Lys Gly Trp Ala Ser 420 425 430 Leu Gln Ser Ile Pro Arg Thr Val Val Leu Asp Thr Lys Thr Gly Ser 440 445 435 Asn Leu Ile Gln Trp Pro Val Val Glu Val Glu Thr Leu Arg Thr Asn 450 455 Ser Thr Asn Leu Gly Ser Ile Ile Val Glu His Gly Ser Val Phe Pro 465 470 475 Leu Ser Leu His Arg Ala Thr Gln Leu Asp Ile Glu Ala Ser Phe Arg 485 490 Leu Asp Pro Leu Asp Val Ala Ala Ala Lys Glu Ala Asp Val Gly Tyr 510 505 Asn Cys Ser Thr Ser Gly Gly Ala Ala Gly Arg Gly Ala Leu Gly Pro 525 515 520 Phe Gly Leu Leu Val Leu Ala Asp Ala Arg Arg His Gly Gly Asp Thr 530 535 540 Glu Gln Thr Ala Val Tyr Phe Tyr Val Ala Arg Gly Leu Asp Gly Asn 545 550 555 Leu Arg Thr His Phe Cys His Asp Glu Ser Arg Ser Ser Arg Ala Asn 565 570 575 Asp Ile Val Lys Arg Val Val Gly Asn Ile Val Pro Val Leu Asp Gly 585 590 Lys Ala Leu Ser Val Arg Val Leu Val Ala His Ser Ile Val Glu Ser 605 600 595 Phe Ala Gln Gly Gly Arg Ser Val Val Thr Ser Arg Val Tyr Pro Thr 610 615 620 Glu Ala Ile Tyr Ala Asn Ala Gly Val Tyr Leu Phe Asn Asn Ala Thr 630 635 640 Gly Ala Arg Val Pro Ala Thr Ser Leu Val Val His Lys Met Asp Pro 645 650 Ser Tyr Asn Gln Asn Gln Ala Glu Met Ala Ser Leu

<210> 88

<211> 473

<212> PRT

<213> Lolium perenne

<400> 88

Val His Trp Arg His Leu Pro Leu Ala Met Val Pro Asp Gln Trp Tyr 10

Asp Ile Asn Gly Val Trp Thr Gly Ser Ala Thr Val Phe Pro Asp Gly 25 Thr Leu Asn Met Leu Tyr Thr Gly Ser Thr Asn Ala Ser Val Gln Ala 45 Gln Cys Leu Ala Val Pro Glu Asp Pro Asn Asp Ser Leu Leu Arg Asn 55 Trp Thr Lys His Glu Ala Asn Pro Val Leu Leu Pro Pro Pro Gly Ile 75 70 Gly Asp Lys Asp Phe Arg Asp Pro Thr Thr Ala Trp Phe Asp Glu Ser 90 95 Asp Gln Thr Trp Arg Thr Val Ile Gly Ser Lys Asp Asn Asn Gly His 105 110 100 Ala Gly Ile Ala Met Val Tyr Lys Thr Lys Asp Phe Leu Asn Tyr Glu 115 120 Leu Ile Pro Gly Tyr Leu His Arg Val Asp Gly Thr Gly Met Trp Glu 140 135 Cys Ile Asp Phe Tyr Pro Val Gly Gly Lys Asn Gly Ser Glu Glu Leu 155 150 Tyr Val Ile Lys Glu Ser Ser Asp Asp Asp Arg His Asp Trp Tyr Thr 165 170 175 Leu Gly Lys Tyr Asp Ala Ala Ala Asn Thr Phe Thr Ala Ala Asp Pro 180 185 190 Glu Asn Asp Leu Gly Ile Gly Leu Arg Tyr Asp Trp Gly Lys Phe Tyr 195 200 205 Ala Ser Lys Thr Phe Tyr Asp Pro Ala Lys Lys Arg Arg Val Leu Trp 215 220 Gly Trp Ile Gly Glu Thr Asp Ser Glu Arg Ala Asp Val Ala Lys Gly 235 230 Trp Ala Ser Leu Met Ser Ile Pro Arg Thr Val Glu Leu Asp Glu Lys 245 250 255 Thr Trp Thr Asn Leu Ile Gln Trp Pro Val Glu Glu Ile Glu Thr Leu 260 265 270 Arg Ile Lys Ser Thr Asp Leu Gly Gly Ile Thr Ile Asp His Gly Ser 275 280 285 Val Tyr Pro Leu Pro Leu His Arg Ala Thr Gln Leu Asp Ile Glu Ala 295 300 Ser Phe Arg Leu Asp Ala Ala Thr Val Ala Ala Leu Asn Glu Ala Asp 305 310 315 Val Gly Tyr Asn Cys Ser Thr Ser Gly Gly Ser Thr His Arg Gly Ala 325 330 335 Leu Gly Pro Phe Gly Ile Leu Val Leu Ala Asp Gly Lys Ala Glu Gln 340 345 Thr Ala Val Tyr Phe Tyr Val Ser Lys Gly Leu Asp Gly Ala Leu Glu 360 365 Thr His Phe Cys His Asp Glu Ser Arg Ser Thr Leu Ala Lys Asp Val 380 375 Val Lys Arg Val Val Gly Tyr Thr Val Pro Val Leu Asp Gly Glu Ala 390 395 Phe Ser Val Arg Val Leu Val Asp His Ser Ile Val Glu Ser Phe Ala 405 410 415 Met Gly Gly Arg Ser Thr Ala Thr Ser Arg Val Tyr Pro Thr Glu Ser 420 425 430 Ile Tyr Gly Ala Ala Gly Ala Tyr Leu Phe Asn Asn Ala Thr Gly Gly 435 440 445 Ser Val Thr Val Glu Lys Leu Val Val His Glu Met Asp Ser Ser Tyr 455 Asn Gln Ile Phe Met Ala Asp Asp Leu 470

<210> 89

<211> 539

<212> PRT

<213> Lolium perenne

PCT/NZ02/00239

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Thr His Ala Ile Pro Lys Ser Ala Ser Gly Lys Ile Leu Arg Lys Glu 520 Leu Arg Ala Lys Leu Ala Ala Pro Ala Thr Ala 535 530 <210> 90 <211> 559 <212> PRT <213> Lolium perenne <400> 90 Met Gly Ser Ile Ala Ala Asp Leu Ala Pro Glu Ala Pro Ala Glu Leu 1.0 Val Phe Arg Ser Lys Leu Pro Asp Ile Glu Ile Pro Thr His Leu Thr 25 Leu Gln Asp Tyr Cys Phe Glu Arg Leu Pro Glu Leu Ser Ala Arg Ala 40 35 Cys Leu Ile Asp Gly Ala Thr Gly Ala Ala Leu Thr Tyr Gly Glu Val 55 Asp Ala Leu Ser Arg Arg Cys Ala Ala Gly Leu Arg Arg Leu Gly Val 70 75 Arg Lys Gly Asp Val Val Met Ala Leu Leu Arg Asn Cys Pro Glu Phe 85 90 Ala Phe Val Phe Leu Gly Ala Ala Arg Leu Gly Ala Ala Thr Thr Thr 110 105 Ala Asn Pro Phe Tyr Thr Pro His Glu Ile His Arg Gln Ala Ala Ala 120 125 115 Ala Gly Ala Lys Val Ile Val Thr Glu Ala Cys Ala Val Glu Lys Val 135 1.40 Arg Ala Phe Ala Ala Glu Arg Gly Ile Pro Val Val Ser Val Asp Glu 145 150 155 Ala Val Asp Asp Gl $ar{ exttt{y}}$ Cys Leu Pro Phe Ala Ala Thr Leu Leu Gly Glu 165 170 175 Glu Ser Gly Glu Arg Phe Val Asp Glu Ala Val Asp Pro Asp Asp Val 185 190 180 Val Ala Leu Pro Tyr Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val 195 200 205 Met Leu Thr His Arg Ser Leu Val Thr Ser Val Ala Gln Gln Val Asp 215 220 Gly Glu Asn Pro Asn Leu His Phe Ser Ser Ser Asp Val Leu Leu Cys 225 230 235 240 Val Leu Pro Leu Phe His Ile Tyr Ser Leu Asn Ser Val Leu Leu Ala 250 Gly Leu Arg Ala Gly Cys Ala Ile Val Ile Met Arg Lys Phe Asp His 270 260 265 Gly Ala Leu Val Asp Leu Val Arg Ala His Gly Val Thr Val Ala Pro 285 275 280 Phe Val Pro Pro Ile Val Val Glu Ile Ala Lys Ser Ala Arg Val Thr 300 295 Ala Ala Asp Leu Ala Ser Ile Arg Leu Val Met Ser Gly Ala Ala Pro 305 310 315 320 Met Gly Lys Glu Leu Gln Asp Ala Phe Met Ala Lys Ile Pro Asn Ala 330 335 325 Val Leu Gly Gln Gly Tyr Gly Met Thr Glu Ala Gly Pro Val Leu Ala 340 345 Met Cys Leu Ala Phe Ala Lys Glu Pro Phe Glu Val Lys Ser Gly Ser 360 355 Cys Gly Thr Val Val Arg Asn Ala Glu Leu Lys Ile Val Asp Pro Asp 380 375 Thr Gly Ala Ser Leu Gly Arg Asn Leu Pro Gly Glu Ile Cys Ile Arg 390 395 400 Gly Lys Gln Ile Met Lys Gly Tyr Leu Asn Asp Pro Glu Ala Thr Lys 410 405 Asn Thr Ile Asp Lys Asp Gly Trp Leu His Thr Gly Asp Ile Gly Tyr 425 420

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Val Asp Asp Asp Glu Ile Phe Ile Val Asp Arg Leu Lys Glu Ile
                      440
Ile Lys Tyr Lys Gly Phe Gln Val Pro Pro Ala Glu Leu Glu Ala Leu
                           460
                  455
Leu Ile Thr His Pro Glu Ile Lys Asp Ala Ala Val Val Ser Met Gln
                                475
    470
Asp Glu Leu Ala Gly Glu Val Pro Val Ala Phe Val Val Arg Thr Glu
                 490 495
            485
Gly Ser Glu Ile Ser Glu Asn Glu Ile Lys Gln Phe Val Ala Lys Glu
        500 505 510
Val Val Phe Tyr Lys Arg Ile Cys Lys Val Phe Phe Ala Asp Ser Ile
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<213> Festuca arundinacea
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                          25
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Leu Gln Asp Tyr Cys Phe Gln Arg Leu Pro Glu His Ser Ala Arg Ala
                    40
       35
Cys Leu Ile Asp Gly Ala Thr Gly Ala Ala Leu Thr Tyr Gly Glu Val
                          60
                 55
Asp Ala Leu Ser Arg Arg Cys Ala Ala Gly Leu Arg Arg Leu Gly Val
                                75
                70
Arg Lys Gly Asp Val Val Met Ala Leu Leu Arg Asn Cys Pro Glu Phe
                            90
            85
Ala Phe Val Phe Leu Gly Ala Ala Arg Leu Gly Ala Ala Thr Thr
         100
                                         110
                         105
Ala Asn Pro Phe Tyr Thr Pro His Glu Ile His Arg Gln Ala Thr Ala
              120
Ala Gly Ala Lys Val Ile Val Thr Glu Ala Cys Ala Val Glu Lys Val
 130 135
                                   140
Arg Ala Phe Ala Ala Glu Arg Gly Ile Thr Val Val Ser Val Asp Glu
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                150
145
Gly Val Asp Asp Gly Cys Leu Pro Phe Gly Glu Thr Leu Leu Gly Glu
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             165
                            170
Asp Gly Gly Glu Arg Phe Val Asp Glu Ala Val Asp Pro Asp Asp Val
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Val Ala Leu Pro Tyr Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val
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 Met Leu Thr His Arg Ser Leu Val Thr Ser Val Ala Gln Gln Val Asp
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 Gly Glu Asn Pro Asn Leu His Phe Ser Ser Ser Asp Val Leu Leu Cys
                              235 240
               230
 Val Leu Pro Leu Phe His Ile Tyr Ser Leu Asn Ser Val Leu Leu Ala
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            245
 Gly Leu Arg Ala Gly Cys Ala Ile Val Ile Met Arg Lys Phe Asp His
                         265
                                           270
          260
 Gly Ala Leu Val Asp Leu Val Arg Ala His Gly Val Thr Val Ala Pro
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 Phe Val Pro Pro Ile Val Val Glu Ile Ala Lys Ser Ala Arg Val Thr 290 295 300
 Ala Ala Asp Leu Ala Ser Ile Arg Leu Val Met Ser Gly Ala Ala Pro
 305 310 315 320
 Met Gly Lys Glu Leu Gln Asp Ala Phe Met Ala Lys Ile Pro Asn Ala
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Val Leu Gly Gln Gly Tyr Gly Met Thr Glu Ala Gly Pro Val Leu Ala 345 Met Cys Leu Ala Phe Ala Lys Glu Pro Phe Glu Val Lys Ser Gly Ser 360 365 Cys Gly Thr Val Val Arg Asn Ala Glu Leu Lys Ile Val Asp Pro Asp 375 Thr Gly Ala Ser Leu Gly Arg Asn Leu Pro Gly Glu Ile Cys Ile Arg 390 395 Gly Lys Gln Ile Met Lys Gly Tyr Leu Asn Asp Pro Glu Ala Thr Lys 410 415 405 Asn Thr Ile Asp Lys Asp Gly Trp Leu His Thr Gly Asp Ile Gly Tyr 425 430 420 Val Asp Asp Asp Glu Ile Phe Ile Val Asp Arg Leu Lys Glu Ile 440 435 Ile Lys Tyr Lys Gly Phe Gln Val Pro Pro Ala Glu Leu Glu Ala Leu 455 460 Leu Ile Thr His Pro Glu Ile Lys Asp Ala Ala Val Val Ser Met Gln 475 470 Asp Glu Leu Ala Gly Glu Val Pro Val Ala Phe Val Val Arg Thr Glu 490 495 485 Gly Ser Glu Ile Ser Glu Asn Glu Ile Lys Gln Phe Val Ala Lys Glu 500 505 Val Val Phe Tyr Lys Arg Ile Cys Lys Val Phe Phe Ala Asp Ser Ile 515 520 Pro Lys Ser Pro Ser Gly Lys Ile Leu Arg Lys Asp Leu Arg Ala Lys 535 540 Leu Ala Ala Gly Ile Pro Gly Ser Glu Thr Thr Gln Ser Lys Ser 555 _. 545 550 <210> 92 <211> 557 <212> PRT <213> Lolium perenne <400> 92 Met Gly Ser Val Pro Glu Glu Ser Val Val Ser Val Ala Ala Glu 10 Thr Val Phe Arg Ser Lys Leu Pro Asp Ile Glu Ile Asn Asn Glu Gln 20 25 Thr Leu Gln Ser Tyr Cys Phe Glu Lys Met Ala Glu Val Ala Ser Arg 40 45 Pro Cys Ile Ile Asp Gly Gln Thr Gly Ala Ser Tyr Thr Tyr Thr Glu 60 55 Val Asp Tyr Leu Thr Arg Arg Ala Ala Ala Gly Leu Arg Arg Met Gly 65 70 Val Gly Lys Gly Asp Val Val Met Asn Leu Leu Arg Asn Cys Pro Glu 85 90 Phe Ala Phe Ser Phe Leu Gly Ala Ala Arg Leu Gly Ala Ala Thr Thr 110 100 105 Thr Ala Asn Pro Phe Tyr Thr Pro His Glu Ile His Arg Gln Ala Glu 115 120 125 Ala Ala Gly Ala Lys Leu Ile Val Thr Glu Ala Cys Ala Val Glu Lys 135 140 Val Leu Glu Phe Ala Ala Gly Arg Gly Leu Pro Val Val Thr Val Asp 155 145 150 Gly Arg Arg Asp Gly Cys Val Asp Phe Ala Glu Leu Ile Ala Gly Glu 170 175 165 Glu Leu Pro Glu Ala Asp Glu Ala Gly Ile Leu Pro Asp Asp Val Val 180 185 190 Ala Leu Pro Tyr Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met 205 195 200 Leu Thr His Arg Ser Leu Val Thr Ser Val Ala Gln Leu Val Asp Gly 210 215 220 Ser Asn Pro Asn Val Cys Phe Asn Lys Asp Asp Ala Leu Leu Cys Leu

230

WO 03/040306 PCT/NZ02/00239

77

250

285

Leu Pro Leu Phe His Ile Tyr Ser Leu His Thr Val Leu Leu Ala Gly

Leu Arg Val Gly Ala Ala Ile Val Ile Met Arg Lys Phe Asp Val Gly 265

Ala Leu Val Asp Leu Val Arg Ala His Arg Ile Thr Ile Ala Pro Phe 280

245

260

Val Pro Pro Ile Val Val Glu Ile Ala Lys Ser Asp Arg Val Gly Ala 290 295 Asp Asp Leu Ala Ser Ile Arg Met Val Leu Ser Gly Ala Ala Pro Met 305 310 315 Gly Lys Asp Leu Gln Asp Ala Phe Met Ala Lys Ile Pro Asn Ala Val 330 335 325 Leu Gly Gln Gly Tyr Gly Met Thr Glu Ala Gly Pro Val Leu Ala Met 345 Cys Leu Ala Phe Ala Lys Glu Pro Phe Lys Val Lys Ser Gly Ser Cys 365 355 360 Gly Thr Val Val Arg Asn Ala Glu Leu Lys Val Val Asp Pro Asp Thr 370 375 380 Gly Ala Ser Leu Gly Arg Asn Gln Pro Gly Glu Ile Cys Val Arg Gly 390 395 Lys Gln Ile Met Ile Gly Tyr Leu Asn Asp Pro Glu Ser Thr Lys Asn 410 415 405 Thr Ile Asp Lys Asp Gly Trp Leu His Thr Gly Asp Ile Gly Leu Val 425 430 420 Asp Asp Asp Asp Glu Ile Phe Ile Val Asp Arg Leu Lys Glu Ile Ile 440 435 Lys Tyr Lys Gly Phe Gln Val Ala Pro Ala Glu Leu Glu Ala Leu Leu 455 460 Leu Thr Asn Pro Glu Val Lys Asp Ala Ala Val Val Gly Val Lys Asp 470 475 Asp Leu Cys Gly Glu Val Pro Val Ala Phe Ile Lys Arg Ile Glu Gly 490 Ser Glu Ile Thr Glu Asn Glu Ile Lys Gln Phe Val Ser Lys Glu Val 505 510 500 Val Phe Tyr Lys Arg Ile Asn Lys Val Tyr Phe Thr Asp Ser Ile Pro 525 515 520 Lys Asn Pro Ser Gly Lys Ile Val Arg Lys Asp Leu Arg Ala Arg Leu 540 535 Ala Ala Gly Ile Pro Thr Glu Val Ala Ala Pro Arg Ser 550 <210> 93 <211> 557 <212> PRT <213> Festuca arundinacea <400> 93 Met Gly Ser Val Pro Glu Glu Ser Val Val Ala Ala Ala Val Ala Glu 10 5 Thr Val Phe Arg Ser Lys Leu Pro Asp Ile Glu Ile Asn Asn Glu Gln 20 25 Thr Leu Gln Ser Tyr Cys Phe Glu Lys Met Ala Glu Val Ala Ser Arg 40 4.5 Pro Cys Ile Ile Asp Gly Gln Thr Gly Ala Ser Tyr Thr Tyr Thr Glu 55 60 Val Glu Ser Leu Thr Arg Arg Ala Ala Ala Gly Leu Arg Arg Met Gly 70 75 Val Gly Lys Gly Asp Val Val Met Asn Leu Leu Arg Asn Cys Pro Glu 85 90 Phe Ala Phe Ser Phe Leu Gly Ala Ala Arg Leu Gly Ala Ala Thr Thr 105 Thr Ala Asn Pro Phe Tyr Thr Pro His Glu Ile His Arg Gln Ala Glu 120 125 Ala Ala Gly Ala Lys Val Ile Val Thr Glu Ala Cys Ala Val Glu Lys 135 140

Val Leu Glu Phe Ala Ala Glu Arg Gly Leu Pro Val Val Thr Val Asp 155 150 145 Gly Lys Arg Asp Gly Cys Val Asp Phe Ala Glu Leu Ile Ala Gly Glu 170 175 165 Glu Leu Pro Glu Ala Glu Glu Ala Gly Ile Leu Pro Asp Asp Val Val 185 190 180 Ala Leu Pro Tyr Ser Ser Gly Thr Thr Gly Leu Pro Lys Gly Val Met 195 200 205 Leu Thr His Arg Ser Leu Val Thr Ser Val Ala Gln Leu Phe Asp Gly 210 215 220 Ser Asn Pro Asn Val Cys Phe Asn Lys Asp Asp Ala Leu Leu Cys Leu 225 230 235 Leu Pro Leu Phe His Ile Tyr Ser Leu His Thr Val Leu Leu Ala Gly 250 255 245 Leu Arg Val Gly Ala Ala Ile Val Ile Met Arg Lys Phe Asp Val Gly 270 260 265 Ala Leu Val Asp Leu Val Arg Ala His Arg Ile Thr Ile Ala Pro Phe 275 280 285 Val Pro Pro Ile Val Val Glu Ile Ala Lys Ser Asp Arg Val Thr Ala 290 295 300 Asp Asp Leu Thr Ser Ile Arg Met Val Leu Ser Gly Ala Ala Pro Met 315 320 310 Gly Lys Asp Leu Gln Asp Ala Phe Met Ala Lys Ile Pro Asn Ala Val 330 335 325 Leu Gly Gln Gly Tyr Gly Met Thr Glu Ala Gly Pro Val Leu Ala Met 340 345 Cys Leu Ala Phe Ala Lys Glu Pro Phe Lys Val Lys Ser Gly Ser Cys 355 360 Gly Thr Val Val Arg Asn Ala Glu Leu Lys Val Val Asp Pro Asp Thr 375 380 Gly Ala Ser Leu Gly Arg Asn Gln Pro Gly Glu Ile Cys Val Arg Gly 385 390 395 400 Lys Gln Ile Met Ile Gly Tyr Leu Asn Asp Pro Glu Ser Thr Lys Asn 410 405 Thr Ile Asp Lys Asp Gly Trp Leu His Thr Gly Asp Ile Gly Leu Val 425 430 420 Asp Asp Asp Glu Ile Phe Ile Val Asp Arg Leu Lys Glu Ile Ile 445 435 440 Lys Tyr Lys Gly Phe Gln Val Ala Pro Ala Glu Leu Glu Ala Leu Leu 450 455 460 Leu Thr Asn Pro Glu Val Lys Asp Ala Ala Val Val Gly Val Lys Asp 465 470 475 Asp Leu Cys Gly Glu Val Pro Val Ala Phe Ile Lys Arg Ile Glu Gly 485 490 Ser Glu Ile Thr Glu Asn Asp Ile Lys Gln Phe Val Ser Lys Glu Val 500 505 510 Val Phe Tyr Lys Arg Ile Asn Lys Val Tyr Phe Thr Asp Ser Ile Pro 515 520 525 520 Lys Asn Pro Ser Gly Lys Ile Leu Arg Lys Asp Leu Arg Ala Arg Leu 530 535 Ala Ala Gly Ile Pro Thr Glu Val Ala Ala Pro Arg Ser 545 550

<210> 94

<211> 501

<212> PRT

<213> Lolium perenne

<400> 94

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 20
 25
 30

 Leu Pro Pro Gly Pro Ser Gly Ala Pro Ile Val Gly Asn Trp Leu Gln
 35
 40
 45

													_	_	_
	50	_	-		Asn	55					60				
Phe 65	Gly	Glu	Val	Phe	Leu 70	Leu	Arg	Met	Gly	Ile 75	Arg	Asn	Leu	Val	Val 80
Val	Ser	Ser	Pro	Glu 85	Leu	Ala	Lys	Glu	Val 90	Leu	His	Thr	Gln	Gly 95	Val
Glu	Phe	Gly	Ser 100	Arg	Thr	Arg	Asn	Val 105	Val	Phe	Asp	Įle	Phe 110	Thr	Gly
Asn	Gly	Gln 115		Met	Val	Phe	Thr 120		Tyr	Gly	Asp	His 125	Trp	Arg	Lys
Met	Arg 130		Ile	Met	Thr	Val 135	Pro	Phe	Phe	Thr	Asn 140	Lys	Val	Val	Ala
Gln 145		Arg	Val	Gly	Trp 150	Glu	Glu	Glu	Ala	Arg 155	Leu	Val	Val	Glu	Asp 160
	Lys	Ala	Asp	Pro 165	Ala	Phe	Ala	Thr	Ala 170	Gly	Thr	Val	Ile	Arg 175	Arg
Arg	Leu	Gln	Leu 180	Met	Met	Tyr	Asn	Asp 185	Met	Phe	Arg	Ile	Met 190	Phe	Asp
Arg	Arg	Phe 195	Glu	Ser	Val	Asp	Asp 200	Pro	Leu	Phe	Asn	Lys 205	Leu	Lys	Ala
Met	Asn 210	Ala	Glu	Arg	Ser	Ile 215	Leu	Ser	Gln	Ser	Phe 220	Asp	Tyr	Asn	Tàr
225					Ile 230					235					240
_	-			245	Thr				250					255	
			260		Lys			265					270		
_		275			Ile		280					285			
	290				Tyr	295					300				
305					Ser 310					315					320
				325	Ser				330					335	
_		_	340		Val			345					350		
		355			Lys		360					365			
	370				Met	375					380				
385					Ser 390					395					400
				405					410					415	
			420		Lys			425					430		
		435			Val		440					445			
	450				Gly	455					460				
465					Pro 470					475					480
Pro	Gly	Gln	Phe	Ser 485	Asn	Gln	Ile	Leu	Lys 490		Ala	Thr	Val	Val 495	Cys
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<211> 505

<212> PRT

<213> Festuca arundinacea

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		35			Ile		40					45			
	50				Asn	55					60				
65					Leu 70					75					80
				85	Leu				90					95	
			100		Thr			105					110		
		115			Val		120					125			
	130				Thr	135					140				
145					Trp 150 Lys					155					160
				165	ьуs Met				170					175	
			180		Met			185					190		
		195			Ser		200					205			
	210				Val	215					220				
225				-	230 Lys					235					240
				245	Lys				250					255	
			260		Ala			265					270		
	_	275			Asp		280					285			
_	290				Thr	295					300				
305					310 Pro					315					320
				325					330					335	
			340		Gln			345				Leu	350 Arg		
Met	Ala	355 Ile	Pro	Leu	Leu	Val	360 Pro		Met	Asn				Ala	Lys
Leu	370 Ala	Gly	Tyr	Asn				Glu	Ser				Val	Asn	Ala
385 Trp	Phe	Leu	Ala				Glu	Gln				Pro	Asp	Glu 415	400 Phe
Arg	Pro	Glu				Glu	Glu				Val	Glu	Ala 430	Ser	Gly
Asn	. Asp				Leu	Pro	Phe			Gly	Arg	Arg	Ser		Pro
Gly				Ala	Leu	Pro	Ile		Gly	Ile	Thr 460	: Ile		Arg	Leu
		. Asr	. Phe	Glu	Leu 470	Thr		Pro	Pro	Gly	Val		Lys	Leu	Asp 480
465 Thr	Thr	Glu	Lys	Gly 485	Gly		Phe	Ser	Leu 490	His		e Leu	Asn	His 495	Ser
Thr	: Ile	. Val	. Ala	Lys	Pro	Arg	Val	. Phe 505							

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Thr Gly Ala Glu Asp Val Val Leu Lys Val Leu Tyr Cys Gly Ile Cys

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His Thr Asp Leu His Gln Thr Lys Asn His Leu Gly Ala Ser Lys Tyr 60 55 Pro Met Val Pro Gly His Glu Val Val Gly Glu Val Val Gly 70 75 Pro Glu Val Ser Lys Tyr Ser Val Gly Asp Val Val Gly Val Gly Val Ile Val Gly Cys Cys His Asp Cys Arg Pro Cys Lys Ala Asn Val Glu 105 100 Gln Tyr Cys Asn Lys Lys Ile Trp Ser Tyr Asn Asp Val Tyr Thr Asp 115 120 125 Gly Lys Pro Thr Gln Gly Gly Phe Ala Ser Ala Met Val Val Asp Gln 135 - 140 Lys Phe Ala Val Lys Ile Pro Ala Gly Leu Ala Pro Glu Gln Ala Ala 150 155 Pro Leu Leu Cys Ala Gly Val Thr Val Tyr Ser Pro Leu Lys His Phe 170 165 Gly Leu Met Thr Pro Gly Leu Arg Gly Gly Ile Leu Gly Leu Gly Gly 185 190 180 Val Gly His Met Gly Val Lys Val Ala Lys Ser Met Gly His His Val 200 205 Thr Val Ile Ser Ser Ser Asn Lys Lys Arg Ala Glu Ala Met Asp Asp 215 220 Leu Gly Ala Asp Ala Tyr Leu Val Ser Ser Asp Glu Ala Gln Met Ala 235 225 230 Ala Ala Met Asp Ser Leu Asp Tyr Ile Ile Asp Thr Val Pro Val Lys 250 245 His Pro Leu Glu Pro Tyr Leu Ala Leu Leu Lys Met Asp Gly Lys Leu 265 270 260 Val Leu Met Gly Val Ile Ala Glu Pro Leu Ser Phe Val Ser Pro Met 280 285 Val Met Leu Gly Arg Lys Thr Ile Thr Gly Ser Phe Ile Gly Ser Ile 295 300 Glu Glu Thr Glu Glu Val Leu Arg Phe Cys Val Glu Lys Gly Leu Thr 305 310 315 Ser Gln Ile Glu Val Val Lys Met Asp Tyr Leu Asn His Ala Leu Glu 325 330 335 Arg Leu Glu Arg Asn Asp Val Arg Tyr Arg Phe Val Val Asp Val Ala 345 350 340 Gly Ser Asn Ile Lys Asp Ala Asp Ala <210> 98

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<212> PRT

<213> Lolium perenne

<400> 98

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Ala Gly Val Ala His Lys Ile Asp Phe Arg Glu Gly Pro Ala Leu Pro
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Val Leu Asp Glu Leu Leu Glu Asp Glu Ala Asn His Gly Thr Phe Asp
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Phe Val Phe Val Asp Ala Asp Lys Asp Asn Tyr Leu Asn Tyr His Gln
                        185
                               190
      180
Arg Leu Met Lys Leu Val Arg Val Gly Gly Leu Leu Gly Tyr Asp Asn
   195
                     200 205
Thr Leu Trp Asn Gly Ser Val Val Leu Pro Ala Asp Ala Pro Met Arg
210 215 220
Lys Tyr Ile Arg Tyr Tyr Arg Asp Phe Val Leu Glu Leu Asn Lys Ala
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            230
Leu Ala Ala Asp Asp Arg Val Glu Ile Cys Gln Leu Pro Val Gly Asp
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Gly Ile Thr Leu Cys Arg Arg Ala Lys
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                                        30
Ser Glu Val Gly His Lys Ser Leu Leu Gln Ser Asp Ala Leu Tyr Gln
 35
            40
Tyr Ile Leu Glu Thr Thr Val Tyr Pro Arg Glu His Glu Cys Met Lys
 50 55 60
Gln Leu Arg Glu Asp Thr Ala Asn His Pro Trp Asn Leu Met Thr Thr
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Ser Ala Asp Glu Gly Gln Phe Leu Asn Leu Leu Ile Lys Leu Ile Gly
                           90
           85
Ala Lys Lys Thr Met Glu Ile Gly Val Tyr Thr Gly Tyr Ser Leu Leu
        100
                                        110
                         105
Ala Thr Ala Leu Ala Ile Pro Glu Asp Gly Thr Ile Leu Ala Met Asp
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      115 120
Ile Asn Arg Glu Asn Tyr Glu Thr Ile Gly Lys Pro Cys Ile Glu Lys
 130 135 140
Ala Gly Val Ala His Lys Ile Asp Phe Arg Glu Gly Pro Ala Leu Pro
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Val Leu Asp Glu Leu Leu Glu Asp Glu Ala Asn His Gly Ser Phe Asp
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Phe Val Phe Val Asp Ala Asp Lys Asp Asn Tyr Leu Asn Tyr His Gln
                         185
                                      190
        180
Arg Leu Met Lys Leu Val Arg Val Gly Gly Leu Ile Gly Tyr Asp Asn
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    195
Thr Leu Trp Asn Gly Ser Val Val Leu Pro Ala Asp Ala Pro Met Arg
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Lys Tyr Ile Arg Tyr Tyr Arg Asp Phe Val Leu Glu Leu Asn Lys Ala
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Leu Ala Ala Asp Asp Arg Val Glu Ile Cys Gln Leu Pro Val Gly Asp
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Gly Ile Thr Leu Cys Arg Arg Ala Lys
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<213> Festuca arundinacea

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Glu Tyr Val Ile Asn Ala Ala Ala Asp Ala Gly Thr Val Arg Arg Val
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Val Phe Thr Ser Ser Ile Gly Ala Ile Thr Met Asp Pro Asn Arg Gly
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                                  140
Pro Asp Val Val Asn Glu Ser Cys Trp Ser Asp Leu Glu Phe Cys
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145 150
Lys Lys Thr Lys Asn Trp Tyr Cys Tyr Gly Lys Ala Val Ala Glu Gln
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Ala Ala Trp Glu Ala Ala Arg Lys Arg Gly Ile Asp Leu Val Val
         180 185 190
Asn Pro Val Leu Val Val Gly Pro Leu Leu Gln Pro Thr Val Asn Ala
                      200 205
Ser Ala Ala His Ile Leu Lys Tyr Leu Asp Gly Ser Ala Lys Lys Tyr
                215
                                  220
Ala Asn Ala Val Gln Ser Tyr Val Asp Val Arg Asp Val Ala Gly Ala
225 230
                              235
His Ile Arg Val Phe Glu Ala Pro Glu Ala Ser Gly Arg Tyr Leu Cys
          245 250
Ala Glu Arg Val Leu His Arg Gly Asp Val Val Gln Ile Leu Ser Lys
        260 265 270
Leu Phe Pro Glu Tyr Pro Val Pro Thr Arg Cys Ser Asp Glu Val Asn 275 280 285
Pro Arg Lys Gln Pro Tyr Lys Met Ser Asn Gln Lys Leu Gln Asp Leu
 290 295 300
Gly Leu Gln Phe Thr Pro Val Asn Asp Ser Leu Tyr Glu Thr Val Lys
305 310 315
Ser Leu Gln Glu Lys Gly His Leu Leu Val Pro Ser Lys Pro Glu Gly
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Leu Asn Gly Val Thr Ala
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<211> 360

<212> PRT

<213> Festuca arundinacea

<400> 102

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Val Asn Phe Asp Leu Pro His Val Ile Ser Glu Ala Pro Gln Phe Pro 235 230 Gly Val Thr His Val Gly Gly Asp Met Phe Lys Glu Val Pro Ser Gly 250 Asp Ala Ile Leu Met Lys Trp Ile Leu His Asp Trp Ser Asp Gln His 265 260 Cys Ala Thr Leu Leu Lys Asn Cys Tyr Asp Ala Leu Pro Ala His Gly 280 285 275 Lys Val Val Leu Val Glu Cys Ile Leu Pro Val Asn Pro Glu Ala Lys 300 295 Pro Ser Ser Gln Gly Val Phe His Val Asp Met Ile Met Leu Ala His 310 315 Asn Pro Gly Gly Arg Glu Arg Tyr Glu Arg Glu Phe Glu Ala Leu Ala 325 330 335 Arg Gly Ala Gly Phe Thr Gly Val Lys Ser Thr Tyr Ile Tyr Ala Asn 340 345 350 Ala Trp Ala Ile Glu Phe Thr Lys 355 360 <210> 103 <211> 360 <212> PRT <213> FLolium perenne <400> 103 Met Gly Ser Thr Ala Ala Asp Met Ala Ala Ser Ala Asp Glu Asp Ala 10 5 Cys Met Phe Ala Leu Gln Leu Ala Ser Ser Ser Val Leu Pro Met Thr 25 20 Leu Lys Asn Ala Ile Glu Leu Gly Leu Leu Glu Ile Leu Val Ala Ala 4.5 35 · 40 Gly Gly Lys Ser Leu Thr Pro Thr Glu Val Ala Ala Lys Leu Pro Ser 60 50 55 Ala Ala Asn Pro Glu Ala Pro Asp Met Val Asp Arg Ile Leu Arg Leu 65 70 75 Leu Ala Ser Tyr Asn Val Val Thr Cys Leu Val Glu Glu Gly Lys Asp 85 90 Gly Arg Leu Ser Arg Ser Tyr Gly Ala Ala Pro Val Cys Lys Phe Leu 105 110 100 Thr Pro Asn Glu Asp Gly Val Ser Met Ala Ala Leu Ala Leu Met Asn 115 120 125 Gln Asp Lys Val Leu Met Glu Ser Trp Tyr Tyr Leu Lys Asp Ala Val 130 135 Leu Asp Gly Gly Ile Pro Phe Asn Lys Ala Tyr Gly Met Ser Ala Phe 150 155 Glu Tyr His Gly Thr Asp Pro Arg Phe Asn Arg Val Phe Asn Glu Gly 170

Met Lys Asn His Ser Ile Ile Ile Thr Lys Lys Leu Leu Glu Leu Tyr 190 185 His Gly Phe Glu Gly Leu Gly Ser Leu Val Asp Val Gly Gly Val 200 205 Gly Ala Thr Val Ala Ala Ile Ala Ala His Tyr Pro Thr Ile Lys Gly 210 215 220 Val Asn Phe Asp Leu Pro His Val Ile Ser Glu Ala Pro Gln Phe Pro 225 230 235 Gly Val Thr His Val Gly Gly Asp Met Phe Lys Glu Val Pro Ser Gly 250 255 245 Asp Ala Ile Leu Met Lys Trp Ile Leu His Asp Trp Ser Asp Gln His 270 260 265 Cys Ala Thr Leu Leu Lys Asn Cys Tyr Asp Ala Leu Pro Ala His Gly 275 280 285 Lys Val Val Leu Val Glu Cys Ile Leu Pro Val Asn Pro Glu Ala Asn 290 295 300 Pro Ser Ser Gln Gly Val Phe His Val Asp Met Ile Met Leu Ala His 310 315

175

Asn Pro Gly Gly Arg Glu Arg Tyr Glu Arg Glu Phe Gln Ala Leu Ala 330 325 Arg Gly Ala Gly Phe Thr Gly Val Lys Ser Thr Tyr Ile Tyr Ala Asn 340 345 Ala Trp Ala Ile Glu Phe Thr Lys 355 360 <210> 104 <211> 360 <212> PRT <213> Festuca arundinacea <400> 104 Met Gly Ser Thr Ala Ala Asp Met Thr Ala Ser Ala Asp Glu Glu Ala 10 1 5 Cys Met Phe Ala Leu Gln Leu Ala Ser Ser Ser Ile Leu Pro Met Thr 20 25 Leu Lys Asn Ala Ile Glu Leu Gly Leu Leu Glu Ile Leu Val Ala Ala 35 40 Gly Gly Lys Ser Leu Thr Pro Thr Glu Val Ala Ala Lys Leu Pro Ser 50 55 Ala Ala Asn Pro Glu Ala Pro Asp Met Val Asp Arg Met Leu Arg Leu 75 65 70 Leu Ala Ser Tyr Asn Val Val Ser Cys Leu Val Glu Glu Gly Lys Asp 95 85 90 Gly Arg Leu Ser Arg Asn Tyr Gly Ala Ala Pro Val Cys Lys Phe Leu 100 105 Thr Pro Asn Glu Asp Gly Val Ser Met Ala Ala Leu Ala Leu Met Asn 120 125 115 Gln Asp Lys Val Leu Met Glu Ser Trp Tyr Tyr Leu Lys Asp Ala Val 130 135 140 Leu Asp Gly Gly Ile Pro Phe Asn Lys Ala Tyr Gly Met Ser Ala Phe 145 150 155 Glu Tyr His Gly Thr Asp Pro Arg Phe Asn Arg Val Phe Asn Glu Gly 165 170 Met Lys Asn His Ser Ile Ile Ile Thr Lys Lys Leu Leu Glu Leu Tyr 180 185 190 Asp Gly Phe Gln Gly Leu Gly Thr Leu Val Asp Val Gly Gly Gly Val 200 205 195 Gly Ala Thr Val Ala Ala Ile Thr Ala His Tyr Pro Ala Ile Lys Gly 210 215 220 Val Asn Phe Asp Leu Pro His Val Ile Ser Glu Ala Pro Pro Phe Pro 235 225 230 Gly Val Thr His Val Gly Gly Asp Met Phe Lys Lys Val Pro Ser Gly 250 245 Asp Ala Ile Met Met Lys Trp Ile Leu His Asp Trp Ser Asp Gln His 270 265 Cys Ala Thr Leu Leu Lys Asn Cys Tyr Asp Ala Leu Pro Ala His Gly 275 280 285 Lys Val Val Leu Val Glu Cys Ile Leu Pro Val Asn Pro Glu Ala Lys 295 300 Pro Ser Ser Gln Gly Val Phe His Val Asp Met Ile Met Leu Ala His 305 310 315 Asn Pro Gly Gly Arg Glu Arg Tyr Glu Arg Glu Phe Glu Ala Leu Ala 325 330 335 Arg Gly Ala Gly Phe Ala Gly Val Lys Ser Thr Tyr Ile Tyr Ala Asn 340 345 Ala Trp Ala Ile Glu Phe Thr Lys 360 <210> 105 <211> 361

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Ile Ala Tyr Leu Thr Tyr Asp Arg Ala Asp Met Ala Phe Ala His Tyr
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Gly Pro Phe Trp Arg Gln Met Arg Lys Leu Cys Val Met Lys Leu Phe
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Ser Arg Arg Arg Pro Glu Thr Trp Leu Ala Val Arg Asp Glu Ser Ala
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Ala Leu Val Arg Ala Val Ala Arg Arg Thr Gly Glu Ser Val Asp Leu
           165 170 175
Gly Glu Leu Ile Phe Lys Leu Thr Lys Asn Val Ile Phe Arg Ala Ala
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                        185
                                190
Phe Gly Ala Gly Ala Val Ala Ala Asp Ala Glu Glu Gly Asp Gly Ala
     195 200
Gly Lys Gln Asp Glu Phe Ile Ala Ile Leu Gln Glu Phe Ser Lys Leu
                215
                                220
Phe Gly Ala Phe Asn Ile Gly Asp Phe Ile Pro Trp Leu Ser Trp Ala
                            235
            230
Asp Pro Gln Gly Ile Asn Val Arg Leu Arg Ala Ala Arg Asn Ala Leu
           245 250 255
Asp Glu Phe Ile Asp Lys Ile Ile Asp Glu His Met Glu Arg Gly Lys
                                270
      260 265
Asn Pro Asp Asp Ala Asp Ala Asp Met Val Asp Asp Met Leu Ala Phe
 275 280
Leu Pro Glu Ala Lys Pro Lys Lys Gly Ala Ala Gly Asp Gly Val Asp
                 295
                        300.
Asp Leu Gln Asn Thr Leu Arg Leu Thr Arg Asp Asn Ile Lys Ala Ile
               310
                              315
Ile Met Asp Val Met Phe Gly Gly Thr Glu Thr Val Ala Ser Ala Ile
    325 330 335
Glu Trp Ala Met Ala Glu Met Met His Ser Pro Asp Asp Leu Arg Arg
  340 345 350
Leu Gln Gln Glu Leu Val Asp Val Val Gly Leu Asp Arg Asn Val Asp
                     360 365
Glu Ser Asp Leu Asp Lys Leu Pro Phe Leu Lys Cys Val Ile Lys Glu
                  375
                                  380
Thr Leu Arg Leu His Pro Pro Ile Pro Leu Leu His Glu Thr Ala
                               395
                390
Glu Asp Cys Val Val Gly Gly Tyr Ser Val Pro Arg Gly Ser Arg Val
                         410
          405
Met Ile Asn Val Tyr Ala Ile Gly Arg Asp Arg Arg Ala Trp Lys Asp
         420 425
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Ala Asp Val Phe Arg Pro Ser Arg Phe Val Gln Gly Glu Gly Glu Ala
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Ala Gly Leu Asp Phe Lys Gly Gly Cys Phe Glu Phe Leu Pro Phe Gly
                                 460
                 455
Ser Gly Arg Arg Ser Cys Pro Gly Met Ala Leu Gly Leu Tyr Ala Leu
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Glu Leu Ala Val Ala Gln Leu Ala His Gly Phe Ser Trp Glu Leu Pro
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Asp Gly Met Lys Pro Ser Glu Leu Asp Met Ser Asp Val Phe Gly Leu
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Gln Phe Leu Gly Asn Pro Val Thr Asn His Val Gln Ser Ala Glu Gln 470 475 His Asn Gln Asp Val Asn Ser Leu Gly Leu Ile Ser Ala Arg Lys Thr 485 490 Ser Glu Ala Ile Asp Ile Leu Lys Leu Met Ser Ser Thr Phe Leu Val 500 505 Ala Leu Cys Gln Ala Ile Asp Leu Arg His Leu Glu Glu Asn Val Lys 515 520 525 Asn Ala Val Lys Asn Cys Val Lys Met Val Ala Arg Lys Thr Leu Ser 530 535 540 Thr Asn Asp Ser Gly His Leu His Ser Ala Arg Phe Cys Glu Lys Asp 550 555 Leu Leu Thr Ile Asp Arg Glu Ala Val Phe Ala Tyr Ala Asp Asp 565 570 Pro Cys Ser Ala Asn Tyr Pro Leu Met Gln Lys Met Arg Ala Val Leu 580 585 590 Val Glu His Ala Leu Ala Asn Gly Glu Ala Glu Arg Asp Val Gln Thr 605 600 Ser Val Phe Ala Lys Leu Ala Ala Phe Glu Gln Glu Leu Arg Ala Val 620 610 615 Leu Pro Arg Glu Val Glu Ser Ala Arg Cys Ala Val Glu Asn Gly Thr 635 625 630 Ala Ala Gln Gln Asn Arg Ile Thr Glu Cys Arg Ser Tyr Pro Leu Tyr 645 650 655 Arg Phe Val Arg Lys Glu Leu Gly Thr Glu Tyr Leu Thr Gly Glu Lys 665 670 660 Thr Arg Ser Pro Gly Glu Glu Val Asp Lys Val Phe Val Ala Met Asn 680 685 Gln Gly Lys His Ile Asp Ala Leu Leu Glu Cys Leu Lys Glu Trp Asn 690 695 Gly Glu Pro Leu Pro Ile Cys <210> 109 <211> 713 <212> PRT <213> Festuca arundinacea

<400> 109

Met Glu Cys Glu Asn Gly His Val Ala Ala Asn Gly Asp Gly Leu Cys 10 15 Val Ala Gln Pro Ala Arg Ala Asp Pro Leu Asn Trp Gly Lys Ala Ala 30 25 Glu Glu Leu Ser Gly Ser His Leu Asp Ala Val Lys Arg Met Val Glu 40 Glu Tyr Arg Arg Pro Val Val Thr Met Glu Gly Ala Ser Leu Thr Ile 60 55 Ala Met Val Ala Ala Val Ala Ala Gly Ala Asp Thr Arg Val Glu Leu 75 70 Asp Glu Ser Ala Arg Gly Arg Val Lys Glu Ser Ser Asp Trp Val Met 90 Asn Ser Met Ala Asn Gly Thr Asp Ser Tyr Gly Val Thr Thr Gly Phe 105 Gly Ala Thr Ser His Arg Arg Thr Lys Glu Gly Gly Ala Leu Gln Arg 125 115 120 Glu Leu Ile Arg Phe Leu Asn Ala Gly Ala Phe Gly Thr Gly Ser Asp 135 140 Gly His Val Leu Pro Ala Ala Thr Thr Arg Ala Ala Met Leu Val Arg 155 150 Val Asn Thr Leu Leu Gln Gly Tyr Ser Gly Ile Arg Phe Glu Ile Leu 165 170 175 Glu Thr Ile Ala Thr Leu Leu Asn Ala Asn Val Thr Pro Cys Leu Pro 180 185 190 Tyr Arg Gly Thr Ile Thr Ala Ser Gly Asp Leu Val Pro Leu Ser Tyr 195 200

Ile	Ala 210	Gly	Leu	Val	Thr	Gly 215	Arg	Pro	Asn	Ser	Val 220	Ala	Thr	Ala	Pro
Asp 225		Ser	Lys	Val	Asn 230		Ala	Glu	Ala	Phe 235	Lys	Ile	Ala	Gly	Ile 240
Gln			Phe	245	Glu				250	Glu				255	
Asn	Gly	Thr	Ala 260	Val	Gly	Ser	Gly	Leu 265	Ala	Ser	Ile	Val	Leu 270	Phe	Glu
Ala	Asn	Ile 275	Leu	Gly	Ile	Leu	Ala 280	Glu	Val	Leu	Ser	Ala 285	Val	Phe	Cys
Glu	Val 290	Met	Asn	Gly	Lys	Pro 295		Tyr	Thr	Asp	His 300	Leu	Thr	His	Lys
Leu 305		His	His	Pro	Gly 310		Ile	Glu	Ala	Ala 315	Ala	Ile	Met	Glu	His 320
Ile	Leu	Glu	Gly	Ser 325	Ser	Tyr	Met	Met	Leu 330	Ala	Lys	Lys	Leu	Gly 335	Glu
Leu	Asp	Pro	Leu 340		Lys	Pro	Lys	Gln 345	Asp	Arg	Tyr	Ala	Leu 350	Arg	Thr
Ser	Pro	Gln 355	Trp	Leu	Gly	Pro	Gln 360		Glu	Val	Ile	Arg 365	Ala	Ala	Thr
Lys	Ser 370		Glu	Arg	Glu	Ile 375		Ser	Val	Asn	Asp 380	Asn	Pro	Leu	Ile
385			Arg		390					395					400
			Val	405					410					415	
Gly	Lys	Leu	Met 420	Phe	Ala	Gln	Phe	Ser 425	Glu	Leu	Val	Asn	Asp 430	Phe	Tyr
Asn	Asn	Gly 435	Leu	Pro	Ser	Asn	Leu 440	Ser	Gly	Gly	Arg	Asn 445	Pro	Ser	Leu
	450		Phe			455					460				
Glu 465	Leu	Gln	Phe	Leu	Gly 470	Asn	Pro	Val	Thr	Asn 475	His	Val	Gln	Ser	Ala 480
			Asn	485					490					495	
			Glu 500					505					510		
		515					520					525			
	530		Ala			535					540				
545			Asn		550					555					560
			Leu	565					570					575	
			Cys 580					585					590		
		595					600					605			
	610		Val			615					620				
625			Pro		630					635					640
			Ala	645					650				,	655	
			660)				665					670		Gly
		675	ò				680					685	.		Ala
Met	Asn 690		Gly	Lys	His	Ile 695		Ala	Leu	Leu	Glu 700	Cys	Leu	Lys	Glu
Trp 705		Gly	Glu	Pro	710		Ile	Суѕ							

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                 25
Phe Tyr Asp Ala Ser Cys Pro Ser Ala Leu Ala Thr Ile Lys Ser Ala
          40
                                     45
35
Val Thr Ala Ala Val Asn Asn Glu Ala Arg Met Gly Ala Ser Leu Leu
 50 55
Arg Leu His Phe His Asp Cys Phe Val Gln Gly Cys Asp Ala Ser Val
               70
                              75
Leu Leu Asn Asp Thr Ala Asn Phe Thr Gly Glu Gln Thr Ala Phe Pro
                           90
Asn Arg Asn Ser Ile Arg Gly Leu Asn Val Ile Asp Asn Val Lys Ala
        100
                       105
                                  110
Gln Val Glu Ala Val Cys Thr Gln Thr Val Ser Cys Ala Asp Ile Leu
 115 120
                                      125
Ala Val Ala Ala Arg Asp Ser Ile Val Ala Leu Gly Gly Pro Ser Tyr
  130 135 140
Thr Val Pro Leu Gly Arg Arg Asp Ser Thr Thr Ala Ser Leu Ser Glu
               150 155 160
Ala Asn Arg Asp Leu Pro Pro Pro Ser Ser Asp Leu Ala Asp Leu Val
                                    175
                            170
             165
Gly Asn Phe Ser Arg Lys Gly Leu Ser Val Thr Asp Met Val Ala Leu
       180 185
Ser Gly Ala His Thr Ile Gly Arg Ala Ala Cys Leu Asn Phe Arg Ser
195 200 205 :
Arg Ile Tyr Gly Glu Ser Asn Ile Ala Pro Ala Tyr Ala Ala Ser Leu
  210 215
                                  220
Gln Ala Asn Cys Pro Gln Ser Ala Pro Asn Gly Asp Gly Thr Leu Ala
                                235 240
              230
Pro Leu Asp Val Ser Thr Pro Asp Ala Phe Asp Asn Ala Tyr Tyr Gly
                            250
                                            255
            245
Asn Leu Leu Ser Gln Gln Gly Leu Leu His Ser Asp Gln Gln Leu Phe
       260 265 270
Asn Gly Gly Ser Thr Asp Ser Leu Val Ser Thr Tyr Ala Ser Asn Ala
      275 280
Ala Gln Phe Ser Gly Asp Phe Ala Ala Ala Met Val Asn Met Gly Asn
 290 295 300
Ile Gly Val Leu Thr Gly Ala Gln Gly Glu Ile Arg Leu Asn Cys Gly
              310
Lys Val Asn
<210> 111
<211> 344
<212> PRT
<213> Lolium perenne
<400> 111
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Lys Ala Thr Val Leu Ala Ala Val Cys Leu Leu His Gly Gly Gly
        20
                           25
Gly Ser Ser Ala Ser Ala Ala Glu Leu Cys Val Ser Tyr Tyr Asp His
  35
                 40
Thr Cys Pro Asp Ala Tyr Lys Ile Val Gln Gly Val Leu Val Glu Ala
                                    60
 50 55
His Lys Ser Asp Pro Arg Ile Phe Ala Ser Leu Ile Arg Leu His Phe
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95

His Asp Cys Phe Val Leu Gly Cys Asp Gly Ser Leu Leu Leu Asp Thr 90 Phe Pro Gly Phe Gln Ser Glu Lys Asp Ala Arg Pro Asn Asn Asn Ser 105 100 Ala Arg Gly Tyr Pro Val Val Asp Ala Ala Lys Ala Ala Leu Glu Lys 120 125 115 Ala Cys Pro Gly Val Val Ser Cys Ala Asp Ile Leu Ala Leu Ala Ala 135 140 Glu Ile Ser Val Gln Leu Ser Gly Gly Pro Gly Trp Gly Val Leu Leu 145 150 155 160 Gly Arg Leu Asp Gly Lys Thr Ser Ser Ile Ala Gly Ala Gln Asn Leu 165 170 Pro Gly Pro Phe Asp Gly Leu Lys Asn Leu Thr Leu Lys Phe Gln Ala 180 185 190 Val Asn Leu Asp Val Thr Asp Leu Val Ala Leu Ser Gly Ala His Thr 200 205 Phe Gly Arg Val Lys Cys Arg Phe Val Thr Asn Arg Leu Tyr Asn Phe 215 220 Ser Gly Thr Asn Gln Pro Asp Pro Thr Leu Asn Ala Ala Tyr Arg Ala 230 235 Phe Leu Ser Thr Arg Cys Pro Arg Asn Gly Asp Ala Asn Ser Leu Asn 245 250 255 Asp Leu Asp Pro Thr Thr Pro Asp Thr Phe Asp Lys Asn Tyr Phe Thr 260 265 270 . Asn Leu Glu Lys Asn Arg Gly Phe Leu Asp Ser Asp Gln Gln Leu Lys 280 285 Ser Asp Pro Gly Ala Leu Thr Thr Thr Ala Pro Ile Val Asp Arg Phe 295 300 Ala Şer Ser Gln Asp Ala Phe Phe Lys Ser Phe Ala Trp Ser Met Ile 305 310 315 320 Lys Met Gly Asn Ile Leu Pro Ile Thr Asp Pro Ser Arg Gly Glu Val 325 330 Arg Lys His Cys Ala Phe Val Asn 340

<210> 112

<211> 326

<212> PRT

<213> Festuca arundinacea

<400> 112

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Ser His Cys Phe Ser Phe Ser Asp Arg Leu Tyr Asn Phe Thr Gly Leu
 195
                      200
Asp Asn Ala Ser Asp Ile Asp Pro Thr Leu Glu Pro Phe Tyr Met Ala
                215
                                 220
Lys Leu Lys Ser Lys Cys Thr Ser Leu Asp Asp Asn Ser Thr Leu Val
              230 235
Glu Met Asp Pro Gly Ser Phe Lys Thr Phe Asp Leu Asp Tyr Phe Lys
            245
                    250
Leu Val Ser Lys Arg Arg Gly Leu Phe His Ser Asp Gly Ala Leu Leu
    260 265 270
Thr Asp Ala Phe Thr Arg Ala Tyr Ile Leu Arg His Ala Thr Gly Ala
 275 280
Phe Lys Asp Glu Phe Phe Ala Asp Phe Ala Val Ser Met Val Lys Met
 290 295 300
Gly Asn Thr Asp Val Leu Thr Gly Ser Gln Gly Glu Ile Arg Lys Lys
305 310 315
Cys Ser Val Val Asn His
<210> 113
<211> 358
<212> PRT
<213> Lolium perenne
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<400> 113

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315

Ser Gln Gly Gln Ile Arg Asn Asp Cys Ser Ala Pro Asn Lys Gly Arg 325 330 335 Thr Asn Asp Asp Leu Pro Trp Ser Val Leu Glu Thr Val Thr Glu Ala 345 Ala Gln Ser Leu Val Leu 355 <210> 114 <211> 344 <212> PRT <213> Lolium perenne <400> 114 Met Thr Thr Met Gly Gly Ser Ser Ile Leu Pro Ala Pro Thr Val Ala 10 Thr Thr Ala Leu Val Leu Ile Val Leu Phe Ala Ser Pro Ala Thr 20 25 Val Ala Lys Gly Ser Gly Leu Ser Val Gly Phe Tyr Lys Lys Leu Cys 40 Pro Lys Ala Glu Lys Val Val Arg Arg Thr Val Thr Lys Ala Phe Glu Lys Glu Pro Gly Thr Pro Ala Asp Ile Ile Arg Leu Phe Phe His Asp 65 60 Cys Phe Val Arg Gly Cys Asp Ala Ser Val Leu Leu Glu Ser Thr Pro 85 90 Gly Arg Met Ala Glu Arg Asp Ser Lys Ala Asn Asn Pro Ser Leu Asp 105 110 100 Gly Phe Glu Val Ile Ser Asp Ala Lys Glu Thr Leu Glu Lys Leu Cys 115 120 125 Pro Gln Thr Val Ser Cys Ala Asp Ile Leu Ala Leu Ala Ala Arg Asp 130 135 140 Gly Ala Tyr Leu Ala Ser Gly Leu Asp Tyr Ala Val Pro Thr Gly Arg 145 150 155 160 Arg Asp Gly Leu Val Ser Lys Glu Asp Glu Val Leu Pro Ser Val Pro 165 170 175 His Pro Asp Phe Asn His Ser Gln Leu Val Glu Asn Phe Thr Ala Lys 180 185 190 Gly Phe Thr Ala Glu Glu Met Val Thr Leu Ser Gly Ala His Thr Ile 195 200 205 Gly Thr Ser His Cys Ser Ser Phe Thr Asp Arg Leu Tyr Asn Phe Ser 210 215 220 Gln Gly Gly Ala Leu Thr Thr Asp Pro Ala Leu Pro Ala Ala Tyr Ala 225 230 235 240 230 Ala Leu Leu Lys Glu Lys Cys Pro Pro Glu Thr Ala Ala Gln Asn Asp 245 250 Thr Thr Met Val Gln Leu Asp Asp Val Thr Pro Phe Val Met Asp Asn 265 270 260 Gln Tyr Tyr Lys Asn Leu Leu Ala Gly Thr Val Pro Leu Gly Ser Asp 280 285 Val Ala Leu Met Glu Ser Pro Asp Thr Ala Ala Leu Val Glu Leu Tyr 290 295 300 Ala Arg Glu Pro Ala Glu Tyr Trp Ala Lys Arg Phe Val Ala Ala Met 305 310 315 Val Lys Val Ser Glu Met Glu Val Leu Thr Gly Ala Glu Gly Glu Ile 330 325 Arg Leu Asn Cys Ser Lys Val Asn 340 <210> 115 <211> 293 <212> PRT <213> Lolium perenne <400> 115 Thr Arg Glu Asn Tyr Tyr Gly Ser Ser Cys Pro Thr Ala Leu Leu Thr

Ile Arg Thr Val Val Thr Thr Ala Val Leu Leu Asp His Arg Met Gly 25 Ala Ser Leu Leu Arg Leu His Phe His Asp Cys Phe Val Gln Gly Cys 40 4.5 Asp Ala Ser Val Leu Leu Asp Asp Thr Ala Gly Phe Thr Gly Glu Lys 50 55 Gly Ala Gly Pro Asn Ala Gly Ser Leu Arg Gly Leu Glu Val Ile Asp 70 75 Lys Ile Lys Met Leu Leu Glu Phe Met Cys Pro Arg Thr Val Ser Cys 90 85 Ala Asp Ile Leu Ala Val Ala Ala Arg Asp Ser Val Val Arg Leu Gly 110 100 105 Gly Pro Ser Trp Ala Val Gln Leu Gly Arg Arg Asp Ala Thr Thr Ala 120 125 Ser Ala Ser Leu Ala Ser Ser Asp Leu Pro Gly Pro Asn Ser Asn Leu 130 135 140 Asn Asp Leu Leu Thr Ala Phe Ser Lys Lys Gly Leu Ser Thr Thr Asp 145 150 155 Met Val Ala Leu Ser Gly Ala His Thr Ile Gly Arg Ala Gln Cys Gln 165 170 175 Asn Tyr Arg Asn Arg Ile Tyr Thr Asp Thr Asp Ile Asp Gly Ala Phe 185 190 180 Ala Ala Ser Leu Arg Gly Gly Cys Pro Gln Ala Gly Gly Asp Gly Asn 195 200 Leu Ala Pro Leu Asp Ala Ser Ser Pro Asn Thr Phe Asp Asn Gly Tyr 210 215 220 Phe Ser Gly Leu Leu Ser Arg Gln Gly Leu Leu His Ser Asp Gln Ala 235 240 230 Leu Tyr Asp Gly Gly Ser Thr Asp Asp Leu Val Arg Thr Tyr Ala Ser 245 250 255 Asn Asn Asp Gln Phe Gly Ser Asp Phe Ala Ala Ala Met Val Lys Leu 260 265 Ser Asn Ile Gly Leu Leu Thr Gly Ser Ser Gly Glu Ile Arg Val Asn 280 275 Cys Arg Ala Val Asn 290 <210> 116 <211> 311 <212> PRT <213> Festuca arundinacea <400> 116 Met Ala Ser Ala Ser Cys Ile Ser Leu Val Leu Leu Val Ala Leu Ala 1 5 10 Ala Thr Ala Ala Ser Ala Gln Leu Ser Ser Thr Phe Tyr Asp Thr Ser 20 25 Cys Pro Arg Ala Leu Ala Thr Ile Lys Ser Gly Val Ala Ala Ala Val 45 40 Ser Ser Asn Pro Arg Met Gly Ala Ser Leu Leu Arg Leu His Phe His 55 60 Asp Cys Phe Val Asn Gly Cys Asp Ala Ser Val Leu Leu Ser Gly Asn 75 Glu Gln Asn Ala Pro Ala Asn Ala Gly Ser Leu Phe Gly Phe Gly Val 90 95 8.5 Ile Asp Asn Ile Lys Thr Gln Leu Glu Gly Ile Cys Lys Gln Thr Val 100 105 110 Ser Cys Ala Asp Ile Leu Thr Val Ala Ala Arg Asp Ser Val Val Ala 125 120 Leu Gly Gly Pro Ser Trp Thr Val Pro Leu Gly Arg Arg Asp Ser Thr 140 130 135 Ser Ala Thr Gly Asn Thr Gly Asp Leu Pro Gly Pro Gly Ser Ser Leu 155 160 145 150 Ala Gln Leu Gln Ala Ala Phe Ala Lys Lys Asn Leu Asn Thr Val Asp

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Met Val Ala Leu Ser Gly Ala His Thr Ile Gly Arg Ala Gln Cys Gln
                          185
Asn Phe Arg Ser Arg Ile Tyr Gly Gly Asp Ser Asn Ile Asn Ala Ala
                     200
                              205
   195
Phe Ala Thr Ser Leu Lys Ala Asn Cys Pro Gln Ser Gly Gly Asn Gly
                                   220
 210 215
Asn Leu Ala Ala Leu Asp Ala Thr Thr Ala Asn Ala Phe Asp Asn Ala
     230 235 240
Tyr Tyr Thr Asn Leu Leu Ser Gln Lys Gly Leu Leu His Ser Asp Gln
    245 250 255
Val Leu Phe Asn Asn Gly Ser Thr Asp Asn Thr Val Arg Asn Phe Ala
                       265 270
Ser Ser Gly Ala Ala Phe Ser Ser Ala Phe Ala Thr Ala Met Ile Lys
 275 280 285
Met Gly Asn Ile Ser Pro Leu Thr Gly Thr Gln Gly Gln Ile Arg Leu
 290 295
Ser Cys Ser Lys Val Asn Ser
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<210> 117
<211> 230
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<213> Lolium perenne
Met Ala Val Ser Glu Leu Glu Val Asp Gly Val Val Phe Pro Pro Leu
Ala Arg Pro Pro Gly Thr Ala His Ala His Phe Leu Ala Gly Ala Gly
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       20
Val Arg Gly Met Glu Leu Gly Gly Asn Phe Ile Lys Phe Thr Ala Ile
                             4.5
                      40
Gly Val Tyr Leu Gln Ala Asp Ala Ala Val Ser Ala Leu Ala Thr Lys
                   55
                                 60
Trp Ala Gly Lys Pro Ala Asp Glu Leu Ala Ala Asp Asn Ala Phe Phe
                               75
              70
Arg Asp Val Val Thr Gly Glu Phe Glu Lys Phe Thr Pro Val Thr Met
            85
                            90
Ile Leu Pro Leu Thr Gly Ala Gln Tyr Ser Glu Lys Val Thr Glu Asn
                   105 110
         100
Cys Val Ala Tyr Trp Lys Ala Val Gly Lys Tyr Thr Asn Ala Glu Ala
          120 125
Ala Ala Val Asp Lys Phe Lys Glu Ala Phe Arg Thr Glu Ser Phe Pro
 130 135
                                    140
Pro Gly Ala Ser Ile Leu Phe Thr His Ser Pro Ala Gly Val Leu Thr
145 150
                              155
Val Ala Phe Ser Lys Asp Ser Ser Val Pro Glu Ser Gly Gly Val Ala
                                              175
            165 170
Ile Glu Asn Arg Pro Leu Cys Glu Ala Val Leu Glu Ser Ile Ile Gly
                         185 190
         180
Glu His Gly Val Ser Pro Ala Ala Lys Leu Ser Leu Ala Thr Arg Val
  195 . 200 205
Ala Glu Leu Leu Asn Glu Ala Ala Pro Val Gly Gln Ala Ala Ala Glu
 210 215
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Pro Val Ser Val Ser Ala
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<213> Festuca arundinacea
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                             10
Ala Arg Pro Pro Gly Thr Ala His Ala His Phe Leu Ala Gly Ala Gly
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<210> 119

<211> 394

<212> PRT

<213> Lolium perenne

<400> 119

Met Ala Ala Thr Met Thr Val Glu Glu Val Arg Lys Ala Gln Arg Ala 1 5 10 Glu Gly Pro Ala Thr Val Leu Ala Ile Gly Thr Ala Thr Pro Ala Asn 20 25 Cys Val Tyr Gln Ala Asp Tyr Pro Asp Tyr Tyr Phe Lys Ile Thr Lys 35 40 Ser Asp His Leu Ala Asp Leu Lys Glu Lys Phe Lys Arg Met Cys Asp 55 Lys Ser Gln Ile Arg Lys Arg Tyr Met His Leu Thr Glu Glu Ile Leu 65 70 Glu Glu Asn Pro Asn Met Cys Ala Tyr Met Ala Pro Ser Leu Asp Ala 90 85 Arg Gln Asp Ile Val Val Val Glu Val Pro Lys Leu Gly Lys Ala Ala 105 110 100 Ala Gln Lys Ala Ile Lys Glu Trp Gly Gln Pro Arg Ser Lys Ile Thr 115 120 125 His Leu Val Phe Cys Thr Thr Ser Gly Val Asp Met Pro Gly Ala Asp 135 Tyr Gln Leu Thr Lys Met Leu Gly Leu Arg Pro Ser Val Lys Arg Leu 150 155 Met Met Tyr Gln Gln Gly Cys Phe Ala Gly Gly Thr Val Leu Arg Leu 165 170 Ala Lys Asp Leu Ala Glu Asn Asn Arg Gly Ala Arg Val Leu Val Val 185 190 Cys Ser Glu Ile Thr Ala Val Thr Phe Arg Gly Pro His Glu Ser His 200 205 Leu Asp Ser Leu Val Gly Gln Ala Leu Phe Gly Asp Gly Ala Ala Ala 215 Val Ile Ile Gly Ala Asp Pro Asp Val Ser Val Glu Arg Pro Leu Phe 230 235 Gln Leu Val Ser Ala Ser Gln Thr Ile Leu Pro Asp Ser Glu Gly Ala 245 250

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Ile Asp Gly His Leu Arg Glu Val Gly Leu Thr Phe His Leu Leu Lys
                           265
Asp Val Pro Gly Leu Ile Ser Lys Asn Ile Glu Arg Ala Leu Glu Glu
                                        285
                       280
Ala Phe Lys Pro Leu Gly Ile Asp Asp Trp Asn Ser Val Phe Trp Val
                    295
Ala His Pro Gly Gly Pro Ala Ile Leu Asp Met Val Glu Ala Lys Val
                310 315 320
Asn Leu Asn Lys Glu Arg Met Arg Ala Thr Arg His Val Leu Ser Glu
             325 330 335
Tyr Gly Asn Met Ser Ser Ala Cys Val Leu Phe Ile Met Asp Glu Met
                          345 350
       340
Arg Lys Arg Ser Ala Glu Asp Gly His Thr Thr Thr Gly Glu Gly Met
   355 360 365
Asp Trp Gly Val Leu Phe Gly Phe Gly Pro Gly Leu Thr Val Glu Thr
  370 375
Val Val Leu His Ser Met Pro Ile Ala Ala
                 390
<210> 120
<211> 196
<212> PRT
<213> Festuca arundinacea
Met Tyr Phe Val Ser Lys Ser Leu Ala Glu Asn Ala Ala Met Asp Tyr
                               10
Ala Lys Glu Asn Gly Val Asp Phe Ile Ser Ile Ile Pro Thr Leu Val
                         2.5
       2.0
Val Gly Pro Phe Leu Ser Ala Gly Met Pro Pro Ser Leu Val Thr Ala
                                4.5
                      40
Leu Ala Leu Ile Thr Gly Asn Glu Ala His Tyr Ser Ile Leu Lys Gln
                    55
                                      60
Val Gln Leu Val His Leu Asp Asp Leu Cys Asp Ser Met Thr Tyr Leu
               70
                                  75
Phe Glu His Pro Asp Ala Asn Gly Arg Tyr Ile Cys Ser Ser His Asp
                               90
             8.5
Thr Thr Ile His Gly Ile Ala Arg Met Leu Lys Glu Arg Phe Pro Glu
                           105
          100
Tyr Asp Ile Pro Gln Lys Phe Pro Gly Ala Asp Asp Asp Leu Gln Pro
              120 125
Ile His Phe Phe Phe Lys Lys Leu Leu Asp His Gly Phe Arg Phe Arg
                    135
                                      140
Tyr Thr Ala Glu Asp Met Phe Asp Ala Ala Val Trp Thr Cys Arg Glu
145 150 155
Lys Gly Leu Ile Pro Leu Gly Ala Glu Gly Ala Gly Gly Pro Ala Ser
             165 170
Ala Ala Gly Lys Leu Gly Ala Val Leu Val Gly Glu Gly Gln Ala Ile
                   185
          180
Gly Ala Glu Thr
      195
<210> 121
<211> 329
<212> PRT
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<400> 121
Met Ala Thr Glu Ala Lys Gly Glu Thr Val Leu Val Thr Gly Ala Ser
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Gly Phe Ile Gly Ser Trp Leu Val Arg Leu Leu Leu Ala Arg Gly Tyr
          20
                           25
Ser Val His Ala Ala Val Leu Asn Pro Asp Asp Lys Ala Glu Thr Asp
                                        4.5
                        40
His Leu Leu Ala Leu Ala Ala Ala Gly Asp Glu Gly Arg Ile Arg
                     55
                                       60
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ccag cagg actg ggtg aact	gggc acct agca aatt gcag cgtc aaaa	ac g at a ac c gc a	tcca cctc tcgg gatg acga ttat	ttcc caat cctc gatc aact	eg ac a ac gc to gg at	cago gato acgo gggg tatt	cgct agtt ggtc gcat	gta tgg gto cat	cgac gcago cggg gaaa cacto	ggc gac gag cga cct	ggct ttcg atca acaa attt	ccac ctgc igggt iggcc attt	gg a gg c ca a tg c	acgat gate actgo gatt gtco	ctcccg cctggt ggtgaa ccgggc ctccgg cttgtg	840 900 960 1020 1080 1140 1200 1214
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Arg		35					40					45				
Ala	50					55					60					
65					Pro 70			•		75					80	
				85	Glu				90					95		
			100		Ser			105					110			
		115			Leu		120					125				
	130				Met	135					140					
145					Phe 150					155					160	
				165	Ala				170					175		
			180		Ala			185				-	190			
		195			Ser		200					205				
	210				Asn	215					220					
225					Pro 230					235					240	
				245	Ile				250					255		
-			260		Leu			265					270			
		275			Gly		280					285				
	290				Thr	295					300					
305					Gly 310					315					320	•
				325	Gly				330					335		
			340		Leu			345					350			
		355			Ala		360					365				
	370				Arg	375					380					
Asp 385	Trp	Gly	Lys	Tyr	Туr 390	Ala	Ser	Lys	Ser	Phe 395		Asp	Gln	ьуѕ	Lys 400	

129

Asn Arg Arg Ile Val Trp Ala Tyr Ile Gly Glu Thr Asp Ser Glu Gln 405 410 Ala Asp Ile Thr Lys Gly Trp Ala Asn Leu Met Thr Ile Pro Arg Thr 425 430 Val Glu Leu Asp Arg Lys Thr Arg Thr Asn Leu Ile Gln Trp Pro Val 440 435 Glu Glu Val Asp Thr Leu Arg Arg Asn Ser Thr Asp Leu Gly Arg Ile 455 460 Thr Val Asn Ala Gly Ser Val Ile Arg Leu Pro Leu His Gln Gly Ala 470 475 Gln Leu Asp Ile Glu Ala Ser Phe Gln Leu Asn Ser Ser Asp Val Asp 490 495 Ala Ile Asn Glu Ala Asp Val Gly Tyr Asn Cys Ser Thr Ser Gly Ala 505 Ala Val Arg Gly Ala Leu Gly Pro Phe Gly Leu Leu Val Leu Ala Asn 520 525 515 Gly Arg Thr Glu Gln Thr Ala Val Tyr Phe Tyr Val Ser Lys Gly Val 535 540 530 Asp Gly Gly Leu Gln Thr His Phe Cys His Asp Glu Ser Arg Ser Thr 550 555 560 Arg Ala Lys Asp Val Val Asn Arg Met Ile Gly Ser Ile Val Pro Val 565 570 575 Leu Asp Gly Glu Thr Phe Ser Val Arg Val Leu Val Asp His Ser Ile 585 Val Gln Ser Phe Ala Met Gly Gly Arg Ile Thr Ala Thr Ser Arg Ala 595 600 605 Tyr Pro Thr Glu Ala Ile Tyr Ala Ala Ala Gly Val Tyr Leu Phe Asn 615 620 Asn Ala Thr Gly Ala Thr Val Thr Ala Glu Arg Leu Val Val His Glu 635 625 ' 630 Met Ala Ser Ala Asp Asn His Ile Phe Thr Asn Asp Asp Leu 645

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<211> 620

<212> PRT

<213> Festuca arundinacea

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<400> 164

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200

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Ala Asn Pro Val Leu Ala His Pro Gln Gly Val Gln Gly Met Asp Phe
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                                  220
Arg Asp Pro Thr Ser Ala Trp Phe Asp Lys Ser Asp Ala Thr Trp Arg
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             230
Ile Leu Ile Gly Ser Lys Asp Asp Asp Asn Gly Ser His Ala Gly Ile
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Ala Phe Ile Phe Lys Thr Lys Asp Phe Leu Ser Phe Glu Arg Val Pro
                        265 270
         260
Gly Ile Val His Arg Val Glu Gly Thr Gly Met Trp Glu Cys Ile Asp
   275 280
Phe Tyr Pro Val Gly Gly His Asn Ser Ser Ser Glu Glu Leu Tyr
                  295
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Val Ile Lys Ala Ser Met Asp Asp Glu Arg His Asp Tyr Tyr Ser Leu
305 310
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Gly Arg Tyr Asp Ala Ala Ala Asn Thr Trp Thr Pro Leu Asp Ala Glu
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Leu Asp Leu Gly Ile Gly Leu Arg Tyr Asp Trp Gly Lys Leu Tyr Ala
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Ala Thr Ser Phe Tyr Asp Pro Leu Lys Gln Arg Arg Ile Met Leu Gly
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Tyr Val Gly Glu Thr Asp Ser Ala Arg Ala Asp Val Ala Lys Gly Trp
 370 375
Ala Ser Leu Gln Ser Ile Pro Arg Thr Val Thr Leu Asp Glu Lys Thr
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385 390
Arg Thr Asn Leu Leu Trp Pro Val Glu Val Glu Ala Leu Arg
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                                           415
           405
Tyr Asn Ser Thr Asp Leu Ser Gly Ile Thr Val Asp Asn Gly Ser Val
                                430
                         425
        420
Phe His Leu Pro Leu His Gln Ala Thr Gln Leu Asp Ile Glu Ala Ser
 435 440 445
Phe Arg Leu Asp Ala Ser Asp Val Ala Ala Ile Asn Glu Ala Asp Val
450 455
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Gly Tyr Asn Cys Ser Ser Ser Gly Gly Ala Ala Ala Arg Gly Ala Ile
      470
                               475
Gly Pro Phe Gly Leu Leu Val His Ala Ala Gly Asp Leu Arg Gly Glu
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                                            495
Gln Thr Ala Val Tyr Phe Tyr Val Ser Arg Ala Leu Asp Gly Thr Leu
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                                        510
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Arg Thr Ser Phe Cys Asn Asp Glu Thr Arg Ser Ser Arg Ala Arg Asp
   515 520
Val Thr Lys Arg Val Val Gly Ser Thr Val Pro Val Leu His Gly Glu
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Ala Leu Ser Met Arg Val Leu Val Asp His Ser Ile Val Gln Ser Phe
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                            555
Ala Met Gly Gly Arg Val Thr Ala Thr Ser Arg Val Tyr Pro Thr Glu
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                           570
Ala Ile Tyr Ala Arg Ala Gly Val Tyr Leu Phe Asn Asn Ala Thr Gly
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<212> PRT

<213> Festuca arundinacea

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 Gly Trp Arg Gly Phe Leu Thr Val Leu Ala Ala Cys Gly Val Val 35
 40
 45

Leu Leu Val Gly Ala Thr Leu Leu Ala Gly Ser Arg Met Gly Gln Ala 55 Gly Asp Gly Glu Gly Asn Thr Asp Glu Asp Gly Ala Gly Phe Pro 75 70 Trp Ser Asn Glu Met Leu Gln Trp Gln Arg Ala Gly Phe His Tyr Gln 85 90 Pro Glu Gly His Phe Met Ser Asp Pro Asn Gly Pro Val Tyr Tyr Arg 100 105 110 Gly Tyr Tyr His Leu Phe Phe Gln Tyr Asn Arg Arg Gly Val Ala Trp 115 120 125 Asp Asp Tyr Ile Glu Trp Gly His Val Val Ser Gln Asp Leu Val His 135 Trp Arg Pro Leu Pro Leu Ala Met Arg Pro Asp His Trp Tyr Asp Lys 145 150 155 Lys Gly Val Leu Ser Gly Thr Ile Thr Val Leu His Asn Gly Thr Leu 170 165 Val Leu Leu Tyr Thr Gly Val Thr Glu Asp Pro Met Ala Glu Ser Gln 185 190 180 Cys Ile Ala Val Pro Thr Asp Pro Asn Asp Pro Leu Leu Arg His Trp 200 205 Thr Lys His Pro Ala Asn Pro Val Leu Ala His Pro Gln Gly Val Gln 210 215 Gly Met Asp Phe Arg Asp Pro Thr Ser Ala Trp Trp Asp Lys Ser Asp 225 230 235 Ala Thr Trp Arg Ile Leu Ile Gly Ser Lys Asp Asp Asp Asn Gly Ser 245 250 255 His Ala Gly Ile Ala Phe Ile Phe Lys Thr Lys Asp Phe Leu Ser Phe 270 260 265 Glu Arg Val Pro Gly Ile Val His Arg Val Glu Gly Thr Gly Met Trp 275 280 Glu Cys Ile Asp Phe Tyr Pro Val Gly Gly His Asn Ser Ser Ser 290 295 300 290 295 Glu Glu Leu Tyr Val Ile Lys Ala Ser Met Asp Asp Glu Arg His Asp 310 315 Tyr Tyr Ser Leu Gly Arg Tyr Asp Ala Ala Ala Asn Thr Trp Thr Pro 325 330 Leu Asp Ala Glu Leu Asp Leu Gly Ile Gly Leu Arg Tyr Asp Trp Gly 345 350 340 Lys Leu Tyr Ala Ser Thr Ser Phe Tyr Asp Pro Val Lys Gln Arg Arg 355 360 365 Ile Met Leu Gly Tyr Val Gly Glu Val Asp Ser Ala Arg Ala Asp Val 375 380 Ala Lys Gly Trp Ala Ser Leu Gln Ser Ile Pro Arg Thr Val Ala Leu 395 390 Asp Glu Lys Thr Arg Thr Asn Leu Leu Leu Trp Pro Val Glu Glu Val 410 405 415 Glu Ala Leu Arg Tyr Asn Ser Thr Asp Leu Ser Gly Ile Thr Ile Asp 420 425 430 Asn Gly Ser Val Phe His Leu Pro Leu His Gln Thr Thr Gln Leu Asp 435 440 Ile Glu Ala Ser Phe Arg Leu Asp Ala Ser Asp Val Ala Ala Ile Asn 450 455 460 Glu Ala Asp Val Gly Tyr Asn Cys Ser Ser Ser Gly Gly Ala Ala Ala 475 470 Arg Gly Ala Leu Gly Pro Phe Gly Leu Leu Val His Ala Ala Gly Asp 485 490 Leu Arg Gly Glu Gln Thr Ala Val Tyr Phe Tyr Val Ser Arg Ala Leu 500 505 Asp Gly Thr Leu Arg Thr Ser Phe Cys Asn Asp Glu Thr Arg Ser Ser 515 520 525 Arg Ala Arg Asp Val Thr Lys Arg Val Val Gly Ser Thr Val Pro Val 535 540 Leu Asp Gly Glu Ala Leu Ser Met Arg Val Leu Val Asp His Ser Ile 555 550 Val Gln Ser Phe Ala Met Gly Gly Arg Thr Thr Ala Thr Ser Arg Val

132

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410

405

Tyr Asn Ser Thr Asp Leu Ser Gly Ile Thr Val Glu Asn Gly Ser Ile 425 430 Phe His Leu Pro Leu His Gln Ala Thr Gln Leu Asp Ile Glu Ala Ser 440 4.3.5 Phe Arg Leu Asp Ala Ser Asp Val Ala Ala Ile Asn Glu Ala Asp Val 450 455 460 Gly Tyr Asn Cys Ser Ser Ser Gly Gly Ala Ala Ala Arg Gly Ala Leu 470 475 Gly Pro Phe Gly Leu Leu Val His Ala Ala Gly Asp Leu Arg Gly Glu 485 490 495 Gln Thr Ala Val Tyr Phe Tyr Val Ser Arg Ala Leu Asp Gly Ser Leu 500 505 510 Arg Thr Ser Phe Cys Asn Asp Glu Thr Arg Ser Ser Arg Ala Arg Asp 515 • 520 525 Val Thr Lys Arg Val Val Gly Ser Thr Val Pro Val Leu Asp Gly Glu 540 535 Val Leu Ala Met Arg Val Leu Val Asp His Ser Ile Val Gln Ser Phe 550 555 Ala Met Gly Gly Arg Val Thr Ala Thr Ser Arg Val Tyr Pro Thr Glu 565 570 Ala Ile Tyr Ala Arg Ala Gly Val Tyr Leu Phe Asn Asn Ala Thr Gly 580 585 590 Ala Ser Val Thr Ala Glu Arg Leu Ile Val His Glu Met Ala Ser Ala 595 600 Val Tyr Asp Glu Thr Val Met Val Lys Asp Ser 615

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<212> PRT

<213> Lolium perenne

<400> 167

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Thr	Glu	Met	Leu 260	Ser	Pro	Arg	Asn	Ser 265	Glu	Asn	Leu	Gly	Asp 270	Asp	Met
Gly	Glu	Ser 275		Gly	Ala	Tyr	Ile 280		Arg	Ile	Pro	Phe 285		Pro	Arg
Glu	Lys 290		Ile	Pro	Lys	Glu 295		Leu	Trp	Pro	His 300	Ile	Gln	Glu	Phe
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				325					330	Pro				335	
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				405					410	Gln				415	
_			420					425		Met			430		
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	450					455				Ser	460				
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			500					505		Pro			510		
		515					520			His		525			
	530					535				Arg	540				
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				645					650					655	
			660					665					670		His
		675					680					685			Gly
	690					695					700				Thr
705					710					715					His 720
				725					730					735	
			740					745					750		Leu
		755	i				760					765	1		Lys
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Val Gln Asn Ala Asp Leu Val Gln Ile Ile Lys Asn Leu Phe Glu Ala
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Ser Arg Lys Glu Lys Ser Ser Gly Ala Val Gly Phe Val Leu Ser Thr
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Ser Arg Ala Ile Ser Glu Thr Leu Thr Phe Leu Thr Ser Gly Gly Ile
            840 845
     835
Gln Thr Thr Glu Phe Asp Ala Phe Ile Cys Ser Ser Gly Ser Asp Leu
       855 860
Cys Tyr Pro Ser Ser Ser Ser Glu Asp Met Leu Ser Pro Thr Glu Leu
             870
                   875
Pro Phe Met Ile Asp Leu Asp Tyr His Ser Gln Ile Glu Tyr Arg Trp
           885 890
Gly Gly Glu Gly Leu Arg Lys Thr Leu Ile Arg Trp Ala Ala Glu Asn
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                              910
         900
Asn Ser Gln Ser Gly Gln Glu Val Val Thr Glu Asp Glu Glu Cys Ser
                                     925
                     920
Ser Thr Tyr Cys Ile Ser Phe Lys Val Lys Asn Thr Glu Ala Val Pro
          935
                         940
Pro Val Lys Asp Leu Arg Lys Thr Met Arg Ile Gln Ala Leu Arg Cys
      950
                             955
His Val Leu Tyr Ser His Asp Gly Ser Lys Leu Asn Leu Ile Pro Leu
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Leu Ala Ser Arg Ser Gln Ala Leu Arg Tyr Leu Tyr Ile Arg Trp Gly
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        980
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Val Glu Leu Ala Asn Met Thr Val Val Val Gly Glu Ser Gly Asp Thr
    995 1000 1005
Asp Tyr Glu Gly Leu Leu Gly Gly Val His Lys Thr Ile Ile Leu Lys
1010 1015 1020
Gly Ser Phe Asn Ala Ala Pro Asn Gln Leu His Ala Ala Arg Ser Tyr
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Ser Leu Glu Asp Val Ile Ser Phe Asp Lys Pro Gly Ile Ala Ser Val
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Val Leu Asn
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Glu Thr Ala Pro Ala Leu Ala Ala Glu Glu Ser Ser Ala Ala Tyr Asn
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Glu Arg Ser Asn Arg Leu Glu Asn Leu Cys Trp Arg Ile Trp Asn Val
            85
                            90
Ser Arg Gln Lys Lys Gln Val Glu Trp Asp Tyr Thr Lys Glu Val Ala
         100
                         105
Arg Arg Lys Leu Glu Gln Glu Leu Gly Ser Arg Glu Ala Ala Glu Asp
                 120 125
     115
Leu Ser Glu Leu Ser Glu Gly Glu Lys Asp Thr Thr Thr Ala Lys Pro
 130 135
                          140
Asp Ala Ala Ala Gln Pro Ser Ala Asp Asp Gly Glu His Gln Gln
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                                155
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Pro Gln Pro Arg Thr Arg Leu Ala Arg Ile Asn Ser Glu Val Arg Leu 170 Val Ser Asp Asp Glu Glu Glu Gln Thr Lys Lys Arg Asn Leu Tyr Ile 180 185 Val Leu Ile Ser Ile His Gly Leu Val Arg Gly Glu Asn Met Glu Leu 200 205 Gly Arg Asp Ser Asp Thr Gly Gly Gln Val Lys Tyr Val Val Glu Leu 215 220 Ala Arg Ala Leu Ala Ala Thr Ala Gly Val His Arg Val Asp Leu Leu 225 230 235 Thr Arg Gln Ile Ser Cys Pro Asp Val Asp Trp Thr Tyr Gly Glu Pro 245 250 Val Glu Met Leu Glu Arg Leu Ser Ser Ala Asp Ala Asp Asp Asp Asp 260 265 270 Gly Glu Gln Ala Gly Gly Gly Ala Tyr Ile Val Arg Leu Pro Cys Gly 280 285 Pro Arg Asp Gln Tyr Ile Pro Lys Glu Glu Leu Trp Pro His Ile Pro 300 295 Glu Phe Val Asp Arg Ala Leu Ser His Val Thr Glu Val Ala Arg Ala 315 305 310 Leu Gly Glu Gln Leu Gln Pro Pro Pro Ser Pro Ala Asp Gly Ala Val 325 330 335 Ala Ala Pro Ile Trp Pro Tyr Val Ile His Gly His Tyr Ala Asp Ala 345 350 340 Ala Glu Val Ala Ala Asn Leu Ala Ser Ala Leu Asn Val Pro Met Val 360 365 Met Thr Gly His Ser Leu Gly Arg Asn Lys Leu Glu Gln Leu Leu Lys 375 Leu Gly Arg Met Pro Gly Pro Glu Ile Gln Gly Thr Tyr Lys Ile Ala 390 395 400 Arg Arg Ile Glu Ala Glu Glu Thr Gly Leu Asp Thr Ala Glu Met Val 405 410 415 Val Thr Ser Thr Lys Gln Glu Ile Glu Glu Gln Trp Gly Leu Tyr Asp
420 425 430 Gly Phe Asp Leu Met Val Glu Arg Lys Leu Arg Val Arg Gln Arg Arg 435 440 Gly Val Ser Ser Leu Gly Arg Tyr Met Pro Arg Met Ala Val Ile Pro 450 455 460 Pro Gly Met Asp Phe Ser Phe Val Glu Thr Gln Asp Thr Ala Asp Gly 470 475 480 Asp Gly Ala Asp Leu Gln Met Leu Ile Ala Pro Asp Lys Ala Lys Lys 485 490 495 Ala Leu Pro Pro Ile Trp Ser Asp Val Leu Arg Phe Phe Thr Asn Pro 505 His Lys Pro Met Ile Leu Ala Leu Ser Arg Pro Asp Pro Lys Lys Asn 515 520 Val Thr Thr Leu Leu Lys Ala Tyr Gly Glu Ser Arg Gln Leu Arg Glu 535 540 Leu Ala Asn Leu Thr Leu Ile Leu Gly Asn Arg Asp Asp Ile Glu Asp 550 555 Met Ala Gly Gly Gly Ala Val Leu Thr Ala Val Leu Lys Leu Ile 565 570 575 Asp Arg Tyr Asp Leu Tyr Gly Gln Val Ala Tyr Pro Lys His His Lys 580 585 590 Gln Thr Asp Val Pro His Ile Tyr Arg Leu Ala Ala Lys Thr Lys Gly 600 Val Phe Ile Asn Pro Ala Leu Val Glu Pro Phe Gly Leu Thr Ile Ile 615 620 Glu Ala Ala Ala Tyr Gly Leu Pro Val Val Ala Thr Lys Asn Gly Gly 630 635 Pro Val Asp Ile Leu Lys Ala Leu His Asn Gly Leu Leu Val Asp Pro 650 655 645 His Ser Ala Glu Ala Ile Thr Gly Ala Leu Leu Ser Leu Leu Ala Glu 660 665 670 Lys Ser Arg Trp Val Glu Cys Arg Arg Asn Gly Leu Arg Asn Ile His 680

137

Arg Phe Ser Trp Pro His His Cys Arg Leu Tyr Leu Ser His Val Ser 700 695 Thr Tyr Cys Asp Gln Pro Ser Pro His Gln Pro Leu Arg Val Pro Leu 710 715 Ala Leu Gly Ser Ser Thr Ser Phe Gly Ala Asp Asp Ser Leu Ser Asp 730 725 Ser Leu Arg Gly Leu Ser Leu Gln Ile Ser Val Asp Ala Ser Ser Asp 740 745 750 Leu Asn Ala Ala Asp Ser Ala Ala Ala Ile Met Asp Ala Leu Arg Arg 755 760 765 Arg Pro Ala Ser Glu Lys Pro Ala Ser Ser Gly Ala Arg Ala Leu Gly 770 775 780 Phe Ala Pro Gly Arg Arg Glu Ser Leu Leu Val Val Ala Val Asp Cys 785 790 795 Tyr Gly Asp Asp Gly Lys Pro Asp Val Glu Gln Leu Lys Lys Ala Ile 805 810 Asp Ala Ala Val Ser Val Gly Glu Cys Ala Gly Ala Lys Gln Gly Tyr 825 830 820 Val Leu Ser Thr Gly Met Thr Ile Pro Glu Ala Ala Glu Ala Ile Lys 840 845 Ala Cys Gly Ala Asp Val Ala Ser Phe Asp Ala Leu Ile Cys Ser Ser 855 Gly Ala Glu Leu Cys Tyr Pro Trp Lys Glu Leu Val Ala Asp Glu Glu 865 870 875 Tyr Ser Gly His Val Ala Phe Arg Trp Pro Gly Asp His Val Lys Ser 885 890 895 Ala Val Pro Arg Leu Gly Ser Met Glu Glu Ile Ala Leu Ala Ile Asp 900 905 910 Arg Pro Ala Ser Ser Val His Cys His Ala Tyr Ala Ala Thr Asp Ala 915 920 925 Ser Lys Val Ser Ile Thr Glu His Tyr Leu <210> 169 <211> 808 <212> PRT <213> Lolium perenne <400> 169 Met Ala Ala Lys Leu Thr Arg Leu His Ser Leu Arg Glu Arg Leu Gly $1 \hspace{1cm} 5 \hspace{1cm} 10 \hspace{1cm} 15$ Ala Thr Phe Ser Ser His Pro Asn Glu Leu Ile Ala Leu Phe Ser Lys 25 Tyr Val His Gln Gly Lys Gly Met Leu Gln Arg His Gln Leu Leu Thr 40 Glu Phe Glu Ala Leu Phe Glu Ala Asp Lys Glu Arg Tyr Ala Pro Phe 50 55 60 Glu Asp Ile Leu Arg Ala Ala Gln Glu Ala Ile Val Leu Pro Pro Trp 75 70 Val Ala Leu Ala Ile Arg Pro Arg Pro Gly Val Trp Asp Tyr Ile Arg 90 Val Asn Val Ser Glu Leu Ala Val Glu Glu Leu Thr Val Ser Glu Tyr 105 Leu Ala Phe Lys Glu Gln Leu Val Asp Glu His Ala Ser Ser Lys Phe 120 125 Val Leu Glu Leu Asp Phe Glu Pro Phe Asn Ala Ser Phe Pro Arg Pro 135 140 Ser Met Ser Lys Ser Ile Gly Asn Gly Val Gln Phe Leu Asn Arg His 150 155 Leu Ser Ser Lys Leu Phe Gln Asp Lys Glu Ser Leu Tyr Pro Leu Leu 165 170 175 Asn Phe Leu Lys Ala His Asn His Lys Gly Thr Thr Met Met Leu Asn 190 180 185

Asp Arg Ile Gln Ser Leu Arg Gly Leu Gln Ser Ala Leu Arg Lys Ala 195 200 205 WO 03/040306 PCT/NZ02/00239 .

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Ala	Pro	Asp	Pro 260	Ala	Ser	Leu	Glu	Lys 265	Phe	Leu	Gly	Thr	Ile 270	Pro	Met
Met	Phe	Asn 275	Val	Val	Ile	Leu	Ser 280	Pro	His	Gly	Tyr	Phe 285	Ala	Gln	Ser
Asn	Val 290	Leu	Gly	Tyr	Pro	Asp 295	Thr	Gly	Gly	Gln	Val 300	Val	Tyr	Ile	Leu
Asp 305		Val	Arg	Ala	Leu 310	Glu	Asn	Glu	Met	Leu 315	Leu	Arg	Ile	Lys	Gln 320
Gln	Gly	Leu	Asp	Ile 325	Thr	Pro	Lys	Ile	Leu 330	Ile	Val	Thr	Arg	Leu 335	Leu
Pro	Asp	Ala	Val 340	Gly	Thr	Thr	Суѕ	Gly 345	Gln	Arg	Leu	Glu	Lys 350	Val	Ile
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Ala	Thr	Leu	Leu 420	Ala	His	Lys	Leu	Gly 425	Val	Thr	Gln	Cys	Thr 430	Ile	Ala
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Lys	Phe 450	Asp	Ser	Gln	Tyr	His 455	Phe	Ser	Cys	Gln	Phe 460	Thr	Ala	Asp	Leu
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Ile	Ala	Gly	Ser	Lys 485	Asp	Ser	Val	Gly	Gln 490		Glu	Ser	His	Ile 495	Ala
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	530					535	Lys				540				
Ile 545	Glu	Glu	Leu	Leu	Tyr 550		Asp	Val	Glu	Asn 555	Ser	Glu	His	Lys	Phe 560
		_	_	565			Pro		570					575	
			580				Gly	585					590		
		595					Asn 600					605			
	610					615					620				
625					630		Lys			635					640
				645			Arg		650					655	
Cys	Asp	Thr	Lys 660		Ala	Phe	Val	Gln 665		Ala	Phe	Tyr	Glu 670	Ala	Phe
Gly	Leu	Thr 675		Val	Glu	Ala	Met 680	Thr	Cys	Gly	Leu	Pro 685		Ile	Ala
	690	His	Gly			695	Glu				700				
705	His	Ile			710		Ser			715					720
		Phe	Glu	Lys 725		Thr	Ala	Asp	Pro 730		Tyr	Trp	Asp	Lys 735	Met

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 Tyr 745
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140

385					390					395	_		3	~ 3	400
			Gly	405					410					415	
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Val	Thr	His 435	Cys	Thr	Ile	Ala	His 440	Ala	Leu	Glu	Lys	Thr 445	Lys	Tyr	Pro
Asn	Ser 450		Leu	Tyr	Trp	Lys 455		Phe	Glu	Asp	His 460		His	Phe	Ser
Cys 465		Phe	Thr	Thr	Asp		Ile	Ala	Met	Asn 475		Ala	Asp	Phe	Ile 480
	Thr	Ser	Thr	Phe 485		Glu	Ile	Ala	Gly 490		Lys	Asp	Thr	Val 495	
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Leu 545		Ser	Leu	His	Pro 550		Ile	Glu	Glu	Leu 555		Tyr	Ser	Asp	Val 560
	Asn	Asp	Glu	His 565		Phe	Val	Leu	Lys 570		Arg	Asn	Lys	Pro 575	
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Val	Val 610		Cys	Gly	Asp	His 615		Asn	Pro	Ser	Lys 620	Asp	Lys	Glu	Glu
Gln 625		Glu	Phe	Lys	Lys 630		Phe	Asp	Leu	Ile 635		Gln	Tyr	Asn	Leu 640
	Gly	His	Ile	Arg 645		Ile	Ser	Ala	Gln 650		Asn	Arg	Val	Arg 655	
Ala	Glu	Leu	Tyr 660		Tyr	Ile	Cys	Asp 665	Thr	Lys	Gly	Ala	Phe 670		Gln
Pro	Ala	Phe 675	Tyr	Glu	Ala	Phe	Gly 680			Val	Ile	Glu 685		Met	Thr
Cys	Gly 690		Pro	Thr	Phe	Ala 695		Ala	Tyr	Gly	Gly 700		Ala	Glu	Ile
Ile 705		Asn	Gly	Val	Ser 710		Tyr	His	Ile	Asp 715	Pro	Tyr	Gln	Gly	Asp 720
	Ala	Ser	Ala	Leu 725		Val	Glu	Phe	Phe	Glu	Lys	Суѕ	Gln	Gly 735	Asp
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Glu	L <u>y</u> s	Tyr 755	Thr	Trp	Lys	Leu	Tyr 760	Ser	Glu	Arg	Leu	Met 765	Thr	Leu	Thr
Gly	Val 770	Tyr	Gly	Phe	Trp	Lys 775		Val	Ser	Asn	Leu 780	Glu		Arg	Glu
Thr 785	Arg		Туг	Leu	Glu 790		Leu	Tyr	Ala	Leu 795	Lys		Arg	Thr	Met 800
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Phe Leu Cys Ala Arg Ala Pro Glu Val Pro Ser Ile Ala Ser Asp Arg Asp Arg 35

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<212> PRT

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His				85					90	Ile				95	
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Pro	Asp	Thr 115	Pro	Gly	Asp	Ile	Lys 120	Gly	Cys	Trp	Ser	Gly 125	Ser	Ala	Thr
Val	Ile 130	Ser	Gly	Ser	Gln	Pro 135	Val	Ile	Met	Tyr	Thr 140	Gly	Gly	Asp	Val
Glu 145	Asn	His	Gln	Val	Gln 150	Asn	Ile	Ala	Leu	Pro 155	Lys	Asn	Arg	Ser	Asp 160
	Tyr	Leu	Ile	Glu 165	Trp	Thr	Lys	Ala	Cys 170	Asn	Asn	Pro	Val	Leu 175	Gln
Pro	Val	Gly	Pro 180		Met	Asn	Pro	Gly 185	Glu	Phe	Arg	Asp	Pro 190	Thr	Thr
Gly	Trp	Ile 195		Pro	Asp	Gly	Leu 200		Arg	Ile	Ser	Ile 205	Gly	Ala	Glu
Val	Asn 210		Tyr	Ser	Ala	Ala 215		Leu	Tyr	Lys	Ser 220	Glu	Asp	Phe	Leu
Asn 225		Ser	Arg	Val	Asp 230	His	Pro	Leu	Tyr	Ser 235	Ser	Ser	Ala	Ser	Thr 240
Met	Trp	Glu	Cys	Leu 245	Asp	Phe	Phe	Ala	Val 250	Leu	Pro	Gly	Ser	Asn 255	Gly
Gly	Leu	Asp	Leu 260	Ser	Ala	Ala	Ile	Pro 265	Lys	Gly	Ala	Lys	His 270	Val	Leu
Lys	Val	Ser 275	۷al	Asp	Gln	Cys	Asp 280	Lys	Tyr	Met	Ile	Gly 285	Val	Tyr	Asp
Leu	Glu 290	His	Asp	Ala	Phe	Val 295	Pro	Asp	Thr	Ile	Leu 300	Asp	Asp	Arg	Trp
Leu 305	Leu	Pro	Arg	Ile	Asp 310	Tyr	Gly	Asn	туг	Tyr 315	Ala	Ser	Lys	Ser	Phe 320
Phe	Asp	Ser	Lys	Asn 325	Arg	Arg	Arg	Ile	Ile 330	Trp	Gly	Trp	Thr	Asn 335	Glu
Ser	Asp	Ser	Ser 340	Ser	Asp	Asp	Val	Ala 345	Lys	Gly	Trp	Ala	Gly 350	Ile	Tyr
Ala	Ile	Pro 355	Arg	Thr	Ile	Trp	Leu 360	Asp	Arg	Asp	Gly	Lys 365	Gln	Leu	Leu
Gln	Trp 370	Pro	Val	Glu	Glu	Ile 375	Glu	Ser	Leu	Arg	Arg 380	Asn	Glu	Ile	Asn
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				405					410	Asp				415	
			420					425		Trp			430		
		435					440			His		445			
	450					455				Met	460				
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465 Cys	Ser	Asp	Ile		470 Arg	Ser	Ser	Leu		475 Gln	Gly	Leu	Tyr		480 Pro
Ala	Tyr	Gly	Gly 500	485 Phe	Phe	Glu	Phe		490 Leu	Glu	Lys	Glu	Arg 510	495 Lys	Ile
Ser	Leu		Thr	Leu	Ile	Asp		505 Ser	Ala	Val	Glu	Ser 525		Gly	Gly
Gly		515 Arg		Cys	Ile		520 Ala	Arg	Val	Tyr	Pro 540	Val	Ala	Ile	Val
Asp 545	530 Asp	Gly	Ser	Ala	His 550	535 Met	Tyr	Ala	Phe	Asn 555			Ser	Thr	Thr 560
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120 Trp Glu Lys Pro Ser Cys Asn Pro Val Ile Pro Tyr Pro Ala Asp Val 130 135 140 Thr Gly Asn Asn Phe Arg Asp Pro Thr Glu Ala Trp Arg Gly Arg Asp 150 155 Gly Leu Trp Arg Val Gly Ile Val Ala Glu Val Lys Gly Val Gly Ser 165 170 175 Leu Leu Val Tyr Arg Ser Ala Asp Phe Leu Arg Trp Gln Arg Asn Ala 185 Ala Pro Leu His Ala Ser Ser Arg Asp Val Pro Val Leu Glu Cys Pro 195 200 Asp Leu Phe Pro Val Ala Ala Ala Gln Gly Ala Thr Glu Gly Leu 210 215 220 Glu Thr Ser Ala Pro Ser Gly Ala Gly Val Arg His Val Leu Lys Leu 230 235 240 Thr Asp Phe Ala Lys Glu Asp His Tyr Met Val Gly Phe Tyr Asp Asp 245 250 255 Val Ala Asp Thr Phe Val Pro Ala Glu Pro Glu Arg Gly Asp Asp Pro 265 Asp Asn Trp Arg Arg Leu Asp His Gly His Leu Tyr Ala Ser Lys Ser 280 Phe Tyr Asp Ala Arg Asn Lys Arg Arg Ile Leu Trp Ala Trp Val Asp 300 295 Glu Thr Asp Gly Gly Gly Val Ala Arg Gly Trp Ala Gly Ile Gln Ala 310 315 320 Phe Pro Arg Ala Met Trp Leu Asp Ala Asp Gly Lys Arg Leu Val Gln 330 325 335 Trp Pro Val Glu Glu Ile Glu Thr Leu Arg Arg Lys Arg Val Gly Leu 345 Arg Trp Ala Thr Asp Val Glu Ala Gly Gly Arg Lys Glu Ile Ala Gly 355 365 360 Ile Val Ser Ser Gln Ala Asp Val Glu Val Val Phe Glu Ile Pro Asn 380 375 Leu Glu Glu Ala Glu Thr Leu Asp Pro Glu Trp Val Leu Asp Pro Lys 395 390 Gly Leu Cys Ala Ala Lys Gly Ala Ser Val His Gly Gly Val Gly Pro 410 Phe Gly Leu Leu Val Leu Ala Ser Gly Asp Leu Glu Glu His Thr Ala 420 425 Val Phe Phe Arg Val Phe Lys His Asp Gly Lys Tyr Lys Val Leu Met

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Ser Tyr Gly Ala Phe Leu Asp Val Asp Val Glu Lys Asp Lys Phe Ile
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           485
Gly Gly Arg Thr Cys Met Thr Ala Arg Val Tyr Pro Glu His Ala Ala
       500 505 510
Met Gly Ser Thr His Leu Tyr Val Phe Asn Asn Gly Thr Gly Ala Val
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Phe Leu Cys Thr Arg Ser Pro Glu Val Pro Ser Ile Ala Ser Lys Arg
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Tyr Arg Thr Ala Tyr His Phe Gln Ser Pro Lys Asn Trp Ile Asn Asp
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Pro Cys Gly Pro Met Tyr Tyr Asn Gly Ile Tyr His Glu Phe Tyr Gln
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                            75
Tyr Asn Pro Gly Gly Thr Ile Ala Ala Asn Ile Val Trp Gly His Ser
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           85
Val Ser Thr Asp Leu Val Asn Trp Ile Gln Leu Glu Pro Ala Ile Val
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                       105 110
Arg Asp Thr Pro Tyr Asp Ile His Gly Cys Trp Thr Gly Ser Ile Thr
                            125
 115 120
Ile Leu Pro Gly Asp Gln Pro Val Ile Ile Tyr Thr Gly Arg Asp Ser
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                                 140
Asp Asn His Gln Ser Gln Asn Ile Glu Leu Pro Lys Asn Arg Ser Asp
145 150 155
Pro Tyr Leu Arg Glu Trp Thr Lys Ala Asp Asn Asn Pro Arg Ile Leu
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Pro Val Gly Pro Asp Leu Asn Leu Thr Gln Phe Arg Asp Pro Thr Thr
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                                       190
Gly Trp Ile Gly Pro Asp Gly Leu Trp Arg Ile Ala Ile Gly Ala Glu
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Leu Asn Gly Tyr Gly Ala Ala Leu Leu Tyr Lys Ser Glu Asp Phe Leu
 210 215
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Asn Trp Thr Arq Val Asp His Pro Leu Tyr Ser Asp Asn Ala Pro Ser
225 230 235 240
Met Trp Glu Cys Pro Asp Phe Phe Ala Val Leu Pro Gly Asn Asn Gly
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Gly Leu Asp Leu Ser Ala Ala Ile Pro Lys Gly Ala Lys His Val Leu
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         260
Lys Met Ser Val Asp Tyr Ser Asp Lys Tyr Met Ile Gly Val Tyr Asp
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Leu Lys Arg Asp Ala Phe Val Pro Asp Val Val Leu Asp Asp Arg Arg
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 290 295
Leu Trp Leu Arg Ile Asp Tyr Gly Thr Phe Tyr Ala Ser Lys Ser Phe
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Phe Asp Ser Lys Arg Gly Arg Arg Val Ile Trp Gly Trp Ser Asn Glu
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WO 03/040306 PCT/NZ02/00239

Thr Asp Ser Val Ser Asp Asp Gly Ala Lys Gly Trp Ala Gly Ile His 345 350 Ala Ile Pro Arg Ser Ile Trp Leu Asp Ser Asp Gly Lys Gln Leu Leu 360 Gln Trp Pro Ile Asp Glu Ile Glu Ser Leu Arg Arg Asp Glu Ile Asn 370 375 380 His Gln Gly Leu Glu Leu Lys Asn Gly Asp Leu Phe Glu Ile Lys Gly 395 400 390 Ile Asp Thr Leu Gln Ala Asp Ile Glu Val Asp Phe Glu Leu Thr Ser 405 410 415 Ile Asp Ser Ala Asp Pro Phe Asp Pro Ser Trp Leu Leu Asp Val Glu 420 425 430 Arg His Cys Arg Glu Ala Gly Ala Ser Val Gln Gly Gly Ile Gly Pro 435 440 445 Phe Gly Leu Val Val Leu Ala Ser Asp Asn Met Glu Glu His Ile Ala 455 460 Val'His Phe Arg Val Tyr Lys Ser Gln Lys Ser His Met Ile Leu Met 470 475 480 Cys Ser Asp Leu Arg Arg Ser Ser Leu Arg Ser Gly Leu Tyr Thr Pro 485 490 495 Ala Tyr Gly Gly Phe Phe Glu Phe Asp Leu Glu Lys Glu Arg Lys Ile 500 505 510 Ser Leu Arg Thr Leu Ile Asp Arg Ser Ala Val Glu Ser Phe Gly Gly 515 520 525 Gly Gly Arg Val Cys Ile Thr Ala Arg Ile Tyr Pro Val Ala Leu Val 535 540 Asp Gly Arg Val His Met Tyr Ala Phe Asn Asn Gly Ser Thr Thr Val 550 555 Arg Val Pro Gln Leu Gly Ala Trp Ser Met Met Thr Ala Gln Val Asn 565 Val Asn Lys Gly · 580 <210> 174 <211> 569 <213> Festuca arundinacea Lolium perenne <400> 174 Met Ala Gln Gly Trp Pro Phe Phe Leu Leu Val Leu Phe Ser Ser Cys 10 15 Val Ser Asn His Leu Val Asn Gly Glu Arg Val Phe Leu Phe Pro Gln 25 Ser His Lys Val Ser Ser Ile Val Ser Lys Arg Tyr Arg Thr Ala Tyr 35 40 His Phe Gln Pro Pro Lys Asn Trp Ile Asn Gly Pro Met Tyr Tyr Asn 60 55 Gly Ile Tyr His Glu Phe Tyr Gln Tyr Asn Pro Asn Gly Ser Leu Trp 70 75 Gly Asn Ile Ile Trp Gly His Ser Val Ser Thr Asp Leu Ile Asn Trp 90 95 Ile Pro Val Glu Pro Ala Ile Glu Arg Asp Ile Pro Ser Asp Ile Asn 100 105 110 Gly Cys Trp Thr Gly Ser Ala Thr Ile Ile Ser Gly Asp Gln Pro Ile 120 125 115 Ile Ile Tyr Thr Gly Ala Asp Lys Glu Asn Arg Gln Leu Gln Asn Ile 140 135 Val Leu Pro Lys Asn Lys Ser Asp Pro Tyr Leu Arg Glu Trp Thr Lys 150 155 Ala Gly Asn Asn Pro Val Ile Gln Pro Val Gly Pro Gly Leu Asn Ala 170 175 165 Ser Gln Phe Arg Asp Pro Thr Thr Gly Trp Ile Gly Pro Asp Gly Leu 180 185 190 Trp Arg Ile Ala Val Gly Ala Glu Leu Asn Gly Tyr Gly Ala Ala Leu 200 205

Leu Tyr Lys Ser Gln Asp Phe Leu Asn Trp Thr Arg Val Asp His Pro

WO 03/040306 PCT/NZ02/00239

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Ser 145	Arg	Pro	Asp	Val	Asn 150	Tyr	Glu	Val	Gln	Asn 155	Val	Ala	Phe	Pro	Lys 160
Asn	Ser	Ser	Asp	Pro 165	Leu	Leu	Arg	Glu	Trp 170	Val	Lys	Pro	Ala	His 175	Asn
Pro	Val	Ile	Val 180	Pro	Glu	Gly	Gly	Ile 185	Asn	Ala	Thr	Gln	Phe 190	Arg ·	Asp
Pro	Thr	Thr 195	Ala	Trp	Tyr	Ala	Asp 200	Gly	His	Trp	Arg	Ile 205	Leu	Val	Gly
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			260					265			•		270	Val	
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	290		_	_	-	295					300			Așn	
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	_			325	-			_	330					335 Gly	
			340					345					350	Ser	
	_	355					360	_		_		365		Arg	
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				405					410	•				415 Ala	
			420				_	425					430	Val	
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	450					455					460			Gly	
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				485					490					495 Phe	
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Gln	Ser	515 Val	Val	Glu	Ser	Phe	520 Gly	Ala	Gly	Gly	Arg	525 Thr	Суз	Ile	Leu
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Pro Thr Thr Ala Trp Phe Asp Glu Ser Asp Gln Thr Trp Arg Thr Val
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Trp Pro Val Glu Glu Ile Glu Thr Leu Arg Ile Lys Ser Thr Asp Leu
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260 265 Ala Leu Val Asp Leu Val Arg Ala His Arg Ile Thr Ile Ala Pro Phe 275 280 285 Val Pro Pro Ile Val Val Glu Ile Ala Lys Ser Asp Arg Val Gly Ala 300 290 · 295 Asp Asp Leu Ala Ser Ile Arg Met Val Leu Ser Gly Ala Ala Pro Met 310 315 Gly Lys Asp Leu Gln Asp Ala Phe Met Ala Lys Ile Pro Asn Ala Val 325 330 335 Leu Gly Gln Gly Tyr Gly Met Thr Glu Ala Gly Pro Val Leu Ala Met 340 345 350 Cys Leu Ala Phe Ala Lys Glu Pro Phe Lys Val Lys Ser Gly Ser Cys 355 360 365 Gly Thr Val Val Arg Asn Ala Glu Leu Lys Val Val Asp Pro Asp Thr 375 380 Gly Ala Ser Leu Gly Arg Asn Gln Pro Gly Glu Ile Cys Val Arg Gly 390 395 Lys Gln Ile Met Ile Gly Tyr Leu Asn Asp Pro Glu Ser Thr Lys Asn 405 410 415 Thr Ile Asp Lys Asp Gly Trp Leu His Thr Gly Asp Ile Gly Leu Val 420 425 430 Asp Asp Asp Glu Ile Phe Ile Val Asp Arg Leu Lys Glu Ile Ile 440 445 435 Lys Tyr Lys Gly Phe Gln Val Ala Pro Ala Glu Leu Glu Ala Leu Leu 455 460 Leu Thr Asn Pro Glu Val Lys Asp Ala Ala Val Val Gly Val Lys Asp 465 470 475 Asp Leu Cys Gly Glu Val Pro Val Ala Phe Ile Lys Arg Ile Glu Gly 485 490 495 Ser Glu Ile Thr Glu Asn Glu Ile Lys Gln Phe Val Ser Lys Glu Val 500 505 510 Val Phe Tyr Lys Arg Ile Asn Lys Val Tyr Phe Thr Asp Ser Ile Pro 520 525 Lys Asn Pro Ser Gly Lys Ile Leu Arg Lys Asp Leu Arg Ala Arg Leu 535 540 Ala Ala Gly Ile Pro Thr Glu Val Ala Ala Pro Arg Ser 550 <210> 179 <211> 501 <212> PRT <213> Lolium perenne <400> 179 Met Glu Val Leu Leu Glu Lys Ala Leu Leu Gly Leu Phe Ala Ala 10 Ala Val Leu Ala Ile Ala Val Ala Lys Leu Ala Gly Lys Arg Phe Arg 25 Leu Pro Pro Gly Pro Ser Gly Ala Pro Ile Val Gly Asn Trp Leu Gln 35 40 Val Gly Asp Asp Leu Asn His Arg Asn Leu Met Gly Ile Ala Lys Arg 50 55 60 Phe Gly Glu Val Phe Leu Leu Arg Met Gly Ile Arg Asn Leu Val Val 70 75 Val Ser Ser Pro Glu Leu Ala Lys Glu Val Leu His Thr Gln Gly Val 90 Glu Phe Gly Ser Arg Thr Arg Asn Val Val Phe Asp Ile Phe Thr Gly 100 105 110 Asn Gly Gln Asp Met Val Phe Thr Val Tyr Gly Asp His Trp Arg Lys 120 125 115 Met Arg Arg Ile Met Thr Val Pro Phe Phe Thr Asn Lys Val Val Ala 130 135 140 Gln Asn Arg Val Gly Trp Glu Glu Glu Ala Arg Leu Val Val Glu Asp 150 155 Val Lys Ala Asp Pro Ala Ser Ala Thr Ala Gly Thr Val Ile Arg Arg

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Cys Ser Glu Ile Thr Ala Val Thr Phe Arg Gly Pro His Glu Ser His 195 200 205 Leu Asp Ser Leu Val Gly Gln Ala Leu Phe Gly Asp Gly Ala Ala Ala 215 220 Val Ile Ile Gly Ala Asp Pro Asp Val Ser Val Glu Arg Pro Leu Phe 225 230 235 240

Ala Lys Asp Leu Ala Glu Asn Asn Arg Gly Ala Arg Val Leu Val Val

185

180

170

175

Gln Leu Val Ser Ala Ser Gln Thr Ile Leu Pro Asp Ser Glu Gly Ala 250

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Pro Gly Leu Val Arg Ala Glu Ala Val Pro Lys Lys Leu Met Ala Leu
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Ser	Asp 210		Leu	Gly	Leu	Leu 215	Gln	Gly	Asp	Thr	Asp 220	Arg	Phe	Thr	Leu
Tyr 225	Gly	Arg	Met	Gly	Tyr 230	Val	His	Ile	Asp	Asp 235	Val	Ala	Arg	Ser	His 240
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	Lys	115	ı				120					125			
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Gln Asn Tyr Arg Asn Arg Ile Tyr Thr Asp Thr Asp Ile Asp Gly Ala
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Phe Ala Ala Ser Leu Arg Gly Gly Cys Pro Gln Ala Gly Gly Asp Gly
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Tyr Phe Ser Gly Leu Leu Ser Arg Gln Gly Leu Leu His Ser Asp Gln
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